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Poster Presentations

T01 | Cell migration

T01-001A

Disrupted-In-Schizophrenia 1 controls microglial movement and phagocytosis

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BACKGROUND: Microglia are the phagocytes of the central nervous system. They survey the micro-environment by migration during early development and by projecting and retracting protrusions at later stages. Appropriate control of microglial movement and phagocytosis is necessary to sculpt and maintain an efficient neuronal network. We study the neurodevelopmental gene Disrupted-In-Schizophrenia 1 (DISC1) as a regulator of microglial functioning. DISC1 disruption is associated with impaired neuronal networks and neurodevelopmental disorders. We hypothesize that loss of DISC1 in microglia impairs cytoskeletal control, disrupts their developmental functions, and contributes to neurodevelopmental disorders.

RESULTS: Our results show that DISC1 is highly expressed in mouse and human microglia. DISC1 locus impairment (LI) microglia phagocytose slower but their final synaptosome uptake is increased compared to wildtype (WT) microglia. DISC1 LI microglia migrate slower compared to WT microglia *in vitro* and in embryonic living brain slices. The relative idling time of *in vitro* and *ex vivo* DISC1 LI microglia is increased and the mean active migration speed is decreased. In contrast, we show that the surveyed brain area of DISC1 LI microglia in adolescent living brain slices is increased compared to WT microglia. Process extension towards laser-induced brain damage seems unchanged. The morphology, ramification index and cell size will be investigated and we are currently validating our findings using a DISC1 LI bone marrow transplantation in WT mice to exclude environmental effects of the DISC1 locus impairment on microglial behavior.

CONCLUSION: DISC1 is a molecular key controlling microglial movement during phagocytosis, migration, and branch motility.

T02 | Cell proliferation, lineages and differentiation

T02-001A

A 4D mouse model of endogenous OPC expansion & its application in demyelinating disease(s)

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Oligodendrocyte Progenitor Cells (OPCs) are homogenously distributed throughout the central nervous system and proliferate throughout life. However, they are characterized by extremely long cell cycles. It has been recognized that there exists a correlation between stem cell differentiation and cell cycle length, and our laboratory has shown that lengthening of the G1 phase is a cause, rather than a consequence, of differentiation. Shortening G1 by overexpression of the cell cycle regulators Cdk4/cyclinD1 (4D) triggers proliferation of neural stem cells during development, adulthood and ageing. On the contrary, it is yet unexplored whether the 4D strategy could be used to trigger proliferation of OPCs.

In this poster, we will introduce an *in vivo* strategy to achieve conditional and temporarily controlled overexpression of 4D using tamoxifen- and doxycycline-inducible systems. Briefly, a triple transgenic mouse was created such that, in oligodendrocyte lineage cells (*Sox10*-creER) tamoxifen triggered the expression of rtTA (*Rosa26*::flox-rtTA-GFP), which followed by doxycycline administration/removal allowed the on/off control of 4D (*TetO*::4D-RFP). Following 3 weeks of 4D overexpression, there was a two-fold increase in OPC proliferation and a significant increase in the density of oligodendrocytes within the corpus callosum. A similar increase in OPC proliferation was observed in the cortical grey matter (Fig.1). This is the first of its kind *in-vivo* expansion of oligodendrocyte lineage cells.

These results have motivated us to investigate the therapeutic potential of 4D-induced cellular amplification in the context of demyelinating disease models (Fig.2). To this aim, a mouse model of demyelination using Cuprizone is being implemented to assess for 4D-induced recovery/rescue of deficits associated with the disease.

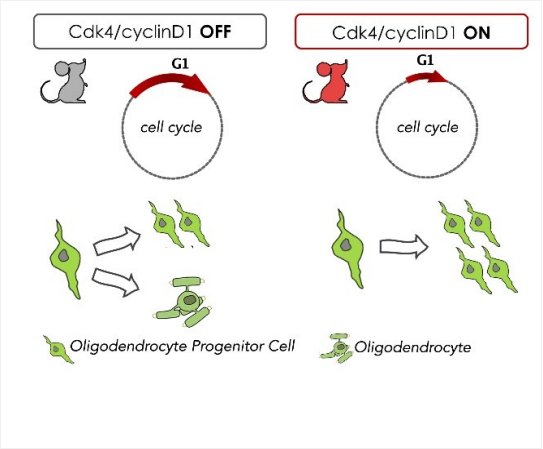

Fig.1: 4D-induced OPC proliferation
Overexpression of Cdk4/cyclinD1 (**4D**) shortens the G1 cell cycle phase and increases proliferation of oligodendrocyte progenitor cells (OPCs).

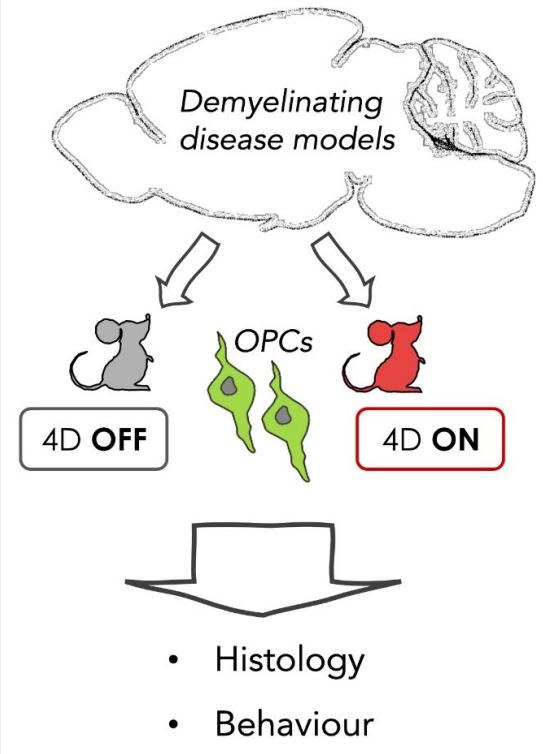

Fig.2: Application of 4D-induced OPC proliferation
Experimental plan to assess the potential of 4D-induced expansion of oligodendrocyte lineage cells in neurodegenerative disease models.

T02-002A

Astrocytes derived from NG2 glia emerge solely following permanent brain ischemia

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NG2 glia display wide proliferation and differentiation potential under physiological and pathological conditions. Here, we examined these two features following different types of brain disorders such as focal cerebral ischemia (FCI), cortical stab wound (SW), and demyelination (DEMY) in 3-month-old animals, in which NG2 glia are labeled by tdTomato under the Cspg4 promoter. To compare NG2 glia expression profiles following different CNS injuries, we employed single-cell RT-qPCR and self-organizing Kohonen map analysis of tdTomato-positive cells isolated from the uninjured cortex/corpus callosum and those after specific injury. Such approach enabled us to distinguish two main populations (NG2 glia, oligodendrocytes), each of them comprising four distinct subpopulations. The expression profiling revealed that a subpopulation of NG2 glia expressing GFAP, a marker of reactive astrocytes, is only present after FCI. However, following less severe injury, namely the cortical SW and DEMY in corpus callosum, subpopulations mirroring different stages of oligodendrocyte maturation markedly prevail. Such injury-dependent incidence of distinct subpopulations was also confirmed by immunohistochemistry. To characterize this unique subpopulation of astrocyte-like NG2 glia, we used single-cell RNA-sequencing analysis and moreover, the patch-clamp technique was employed to disclose their basic membrane properties. Overall, we have proved that astrocyte-like NG2 glia are a specific subpopulation of NG2 glia only emerging following FCI. These cells, located in the ischemic glial scar, are active in the cell cycle and display a current pattern similar to that identified in cortical astrocytes. Thus astrocyte-like NG2 glia may represent important players in glial scar formation and repair processes, following ischemia.

Acknowledgement
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T02-003A

Extensive transcriptional and chromatin changes underlie astrocyte maturation in vivo and in culture

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Astrocytes have diverse functions in brain homeostasis. Many of these functions are acquired during late stages of differentiation when astrocytes become fully mature. However, the mechanisms underlying astrocyte maturation are not well understood.
Using single cell RNA-Seq and other genome-wide analyses, we identified extensive transcriptional changes that occur during murine astrocyte maturation, which were accompanied by chromatin remodelling at regulatory elements. Investigating astrocyte maturation in a cell culture model revealed that in vitro-differentiated astrocytes lacked expression of many mature astrocyte-specific genes, including genes for the transcription factors Rorb, Dbx2, Lhx2 and Fezf2. These factors have been found previously to regulate neuronal subtype specification, but their function in astrocytes is unknown. Forced expression of these factors in vitro induced distinct sets of mature astrocytes-specific transcripts and chromatin remodelling, and it revealed that Fezf2 regulates astrocytic calcium responses. Culturing astrocytes with FGF2 in a three-dimensional gel induced expression of Rorb, Dbx2 and Lhx2 and improved their maturity based on transcriptional and chromatin profiles.
In summary, with this comprehensive characterisation of the transcriptional and chromatin changes underlying astrocyte maturation, which we will provide as an online resource to the community, we have shown that extrinsic signals orchestrate the expression of multiple intrinsic regulators controlling these changes.

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Lattke M, Goldstone R, Guillemot F: *Extensive transcriptional and chromatin changes underlie astrocyte maturation in vivo and in culture*, bioRxiv 2020; doi: https://doi.org/ 10.1101/2020.04.28.066043;

T02-004A

Oligodendrocyte progenitors sense time by creating distinct epigenetic landscapes

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Oligodendrocyte progenitor cells (OPCs) are the fourth glial cell type that during development can give rise to oligodendrocytes (OLs) but in the adult, can be stimulated by learning and cognitive function and tends to regenerate new myelinating cells for the repair of damage in disease. The oligodendrocyte progenitors in the adult brain (aOPCs) comprise 5-8 % of the total brain cells and are uniformly distributed in the brain’s gray and white

matter [1]. While very little is still known about aOPCs, we know they are a different cell type from the neonatal progenitors (nOPCs). Adult OPCs share with nOPCs the immunoreactivity for progenitor markers, such as the expression of PDGFRA, although they are functionally characterized by slower rate of proliferation and migration and unique responsiveness to external stimuli [2]. Since cell identity is modulated by the interplay between epigenetic factors and transcription factors, this work focuses on defining the epigenetic landscapes of nOPCs and aOPCs.

Acknowledgement

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T02-005A

Nerve growth factor promotes differentiation and protection of oligodendrocyte precursor cells from oxygen-glucose deprivation.

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Nerve growth factor (NGF) is a pleiotropic molecule acting on different cell types in physiological and pathological conditions. However, the effect of NGF on the survival and differentiation/maturation of oligodendrocyte precursor cells (OPCs) and oligodendrocytes (OLs), the cells responsible for myelin formation, turnover, and repair in the central nervous system (CNS), is still poorly understood and heavily debated. Here we used mixed neural stem cell (NSC)-derived OPC/astrocyte cultures to clarify the role of NGF throughout the entire process of OL differentiation and investigate its putative role in OPC protection under pathological conditions (Figure 1A-D).

We first showed that the production and gene expression of *Ngf* and its high- and low-affinity receptors (*trkA* and $p75^{NTR}$, respectively) are dynamically regulated during the differentiation process. In particular, the induction of the differentiation by thyroid hormone boosts the expression of *trkA* (Figure 1E-L) and production of NGF, mainly produced by astrocytes.

Since the specificity of the detection of the NGF receptors through an antibody-based assay is highly debated, we used functional experiments to investigate the role of NGF and trkA-mediated pathways in the OPC differentiation. We used NGF protein immunoneutralization and a specific trkA antagonist (GW-441756) to block the NGF action or the trkA activated signaling. The NGF neutralization and the low dose of trkA antagonist (0.1 μM) do not affect cell viability, while highest concentrations of GW-441756 (1 and 10 μM) produced an increase in the percentage of

condensed nuclei. Moreover, both inhibitory strategies are translated to a reduction in OPC differentiation, quantified as an increased percentage of OPCs (NG2-positive cells) at and a reduction of mature oligodendrocytes (CNPase- and MBP-positive cells).

Fetal-derived OPCs are highly sensitive to oxygen-glucose deprivation (OGD) a pathological condition which induces an increase of NGF production in the culture, suggesting a putative role in the response to this noxious stimulus. The hypothesis is also corroborated by the demonstration that NGF treatment in basal condition increases the percentage of mature OLs.

Thus, we tested NGF as a possible treatment to overcome the OGD-mediated toxicity and differentiation impairment, showing that exposure to NGF, both as soluble molecule or as astrocyte-conditioned medium, exerts a protective role.

This study demonstrated that NGF is implicated in OPC differentiation, maturation, and protection in the presence of metabolic challenges, also suggesting implications for the treatment of demyelinating lesions and diseases.

Acknowledgement

The study has been partially supported by the ARSEP Foundation as part of the "Role of RXRγ in T3-mediated oligodendrocyte differentiation and remyelination" project (to LC and VAB) and fellowship (to VAB), MIUR, National Technology Clusters, and project IRMI (CTN01_00177_888744), MIUR (LC).

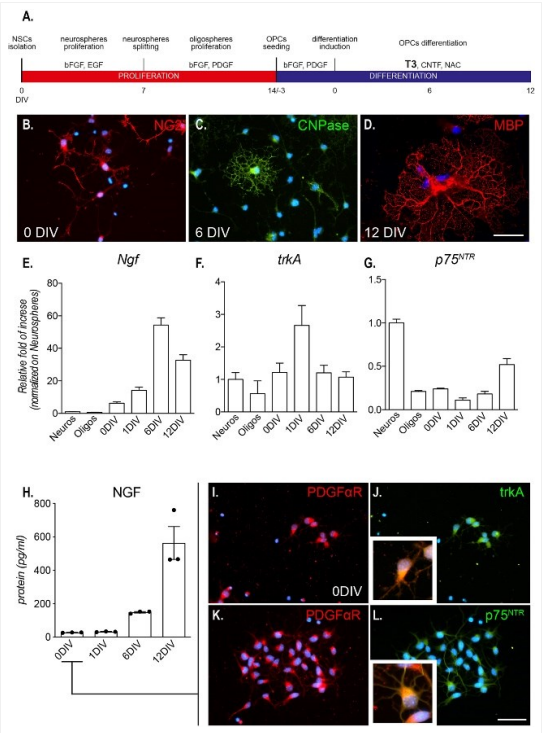

Figure 1. Neural stem cells-derived oligodendrocyte precursor cells / astrocytes mixed culture

(A) Mixed OPC/astrocyte cultures were obtained from Neural Stem Cells.

(B - D) Representative images of culture progress from OPCs (NG2-positive cells, 0 DIV, B), through mature OLs (CNPase-positive cells, 6 DIV, C) to mature/myelinating OLs (CNPase/MBP-positive cells, 12 DIV, D). White bar: 20 µm.

(E-G) Relative mRNA expression normalized on neurospheres.

(H) NGF protein quantification.

(I – L) Representative images of OPCs (PDGFαR-positive cells, I and K) double positive for trkA (J) and $p75^{NTR}$ (L), prior to the differentiation induction mediated by T3 (0 DIV). White bar: 20 µm.

T02-006A

Understanding the proliferative dynamics of microglia in the developing brain.

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Microglia are the resident immune cells of the brain, with many important functions including immuno-vigilance, neuronal maintenance and homeostasis. However, there are many gaps in our knowledge with regard to the spatiotemporal dynamics of these cells during development. The aim of the current study is to examine and characterise the proliferative dynamics of microglia during embryonic and postnatal development. We show that the density of microglia from the forebrain, midbrain and hindbrain steadily increases during embryogenesis and undergoes an exponential growth in the postnatal phase of development. Spatial analysis revealed that during development microglia are found as clusters throughout the brain primordium until the typical 'tiling' distribution of microglia is achieved during the late postnatal stage. We hypothesised that microglia clonally expand during development, which facilitates the rapid increase in cell density and accounts for the clustered formation of microglia during development. In order to test this hypothesis, we have developed a sparse-labelling approach for fate-mapping early embryonic microglia using *Cx_3cr1^{CreER}; $Rosa^{mTmG}$* mice. Briefly, pregnant *Cx_3cr1^{CreER}; $Rosa^{mTmG}$* dams were pulsed with a low dose of tamoxifen at E10.5 labelling a small percentage of the Cx3cr1$^+$ population with a GFP tag. At E11.5, less than 10% of microglia were tagged with GFP and a large proportion of these initially labelled cells were proliferating. Subsequent analysis at E16.5 showed a steady increase in the density of labelled microglia and by P30, very large clusters of labelled microglia were distributed throughout the brain. Therefore, this suggests that the originally labelled microglia have clonally expanded during development. However, due to the nature of a single colour reporter it was difficult to examine other features of clonality from labelled microglia. In order to overcome these limitations, RGB marking with lentiviral vectors was employed to study the clonal dynamics of microglia during postnatal development. Due to the high number of stochastic potential colour combinations, RGB marking is well suited for studying clonality in cells as it allows for a greater degree of clonal discrimination. Analysis at P21 and P30 revealed the presence of microglia clones ranging in size from 1 cell to 49 cells. Interestingly, microglia in larger clones remained spatially related while being integrated into the unique 'tiled' network of microglia, whereas cells from smaller clones were more dispersed from each other. Altogether these findings indicate that clonal expansion is an important process for the rapid colonisation of microglia during development.

T02-007A

The role of MHC-II in efficient OPC differentiation and remyelination

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Multiple sclerosis (MS) is an immune-mediated inflammatory disease of the central nervous system (CNS), characterised by demyelination, oligodendroglial loss and axonal injury. While there are no treatments targeting

myelin repair, research has identified a role for regulatory T (Treg) cells in myelin regeneration[1]; the mechanisms of which remain somewhat unclear. It is however recognised that Treg activation is dependent on MHC-II-T cell receptor (TCR) interactions[2].Therefore, we hypothesised that MHC-II would be required for Treg-mediated OPC differentiation and remyelination. To investigate this requirement for MHC-II, we used *in vitro* pure OPC cultures and an *in vivo* model of lysolecithin-induced demyelination in WT and MHC-II-deficient mice. Surprisingly, we found that Treg cells significantly drive OPC differentiation ($Olig2^+MBP^+$ cells, 9 days *in vitro*) independent of MHC-II *in vitro*—an effect that was comparable to that of MHC-II-expressing WT mice. Immunofluorescent staining of spinal cord sections revealed the absence of MHC-II does not significantly affect the number of oligodendrocyte lineage cells ($Olig2^+$), proliferating OPCs ($Olig2^+Ki67^+$) and differentiated oligodendrocytes ($Olig2^+CC1^+$ cells) following demyelination. There was also no effect on lesion size, indicating MHC-II deficiency does not increase the burden of demyelination. Together, these findings suggest a novel MHC-II-independent mechanism for Tregs in efficient OPC differentiation. Ongoing work is investigating whether MHC-II is required for remyelination *in vivo* and the mechanism(s) by which Tregs function beyond what is classically known in the context of regeneration.

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T02-008A

c-Jun N-terminal Kinase 1 (JNK1) modulates oligodendrocyte progenitor cell architecture, proliferation and myelination

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During Central Nervous System ontogenesis, myelinating oligodendrocytes (OLs) arise from highly ramified and proliferative precursors called oligodendrocyte progenitor cells (OPCs). OPC architecture, proliferation and oligodendro-/myelino-genesis are finely regulated by the interplay of cell-intrinsic and extrinsic factors. A variety of extrinsic cues converge on the extracellular signal-regulated kinase/mitogen activated protein kinase (ERK/MAPK) pathway. Here we found that the germinal ablation of the MAPK c-Jun N-Terminal Kinase isoform 1 (JNK1) results in a significant reduction of myelin in the cerebral cortex and corpus callosum at both postnatal and adult stages.

Myelin alterations are accompanied by higher OPC density and proliferation during the first weeks of life, consistent with a transient alteration of mechanisms regulating OPC self-renewal and differentiation. JNK1 KO OPCs also show smaller occupancy territories and a less complex branching architecture *in vivo*. Notably, these latter phenotypes are recapitulated in pure cultures of JNK1 KO OPCs and of WT OPCs treated with the JNK inhibitor D-JNKI-1. Moreover, JNK1 KO and WT D-JNKI-1 treated OLs, while not showing overt alterations of differentiation *in vitro*, display a reduced surface compared to controls. Our results unveil a novel player in the complex regulation of OPC biology, on the one hand showing that JNK1 ablation cell-autonomously determines alterations of OPC proliferation and branching architecture and, on the other hand, suggesting that JNK1 signaling in OLs participates in myelination *in vivo*.

T02-009B

Analysis of the molecular and functional roles of cortistatin on the dynamics of oligodendrocyte generation and myelination

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Oligodendrocytes (OLs) are the predominant myelinating cells of the central nervous system (CNS). In the developing CNS, oligodendrocyte precursor cells (OPCs) proliferate, migrate, and become mature, acquiring their typical biochemical profile and morphology, forming myelin sheaths. However, OLs may die in different pathologies as in multiple sclerosis (MS). It is a chronic immune-mediated disease characterized by autoimmune inflammation, myelin depletion and axonal degeneration. In the early stages of MS, OLs are actively replaced by OPCs which will mature producing spontaneous remyelination. However, a decrease in the efficiency of myelin regeneration is observed in chronic disease. Therapies approved for MS dampen the immune system's activity but they cannot undo existing myelin damage or replace the lost of myelin sheaths. Identifying the key mechanisms that regulate remyelination is a priority in MS research. Accordingly, special attention has been paid to the identification of molecular factors and cellular mechanisms involved in OL dynamics (proliferation, migration and maturation). Cortistatin (CST) is a neuropeptide with immunomodulatory functions distributed in the nervous and immune systems. Recently, we demonstrated the therapeutic effect of CST in the experimental autoimmune encephalomyelitis murine model of MS by targeting the inflammatory and autoimmune components of this disease. However its role in glial cell dynamics during de-and remyelination is unknown. Using transcriptomic analysis of OLs isolated from CST-deficient transgenic mice, we observed that the lack of CST affected critical molecular pathways of OLs lineage by increasing gene expression correlated with a proliferative and immature profile and by reducing the gene signature of myelinating OLs. These results were functionally validated by in vitro and in vivo assays. OPCs/OL cell cultures revealed a role for CST in cell dynamics, mainly favoring OL differentiation processes. In addition, co-culture of dorsal root ganglia neurons and OLs demonstrated that CST increased myelinating activity of mature OL. Furthermore, when using the demyelinating cuprizona (CPZ) intoxicant model, CST seemed to modulate the time-course of OL development throughout the course of the disease. Indeed, lack of CST was associated with aberrant myelin patterns as observed in the corpus callosum of CPZ mice. This atypical myelin from CST-deficient mice was associated with unsuccessful phagocytosis by microglia. Together, these results indicate that CST is a novel factor that influences OL cell fate and suggest that CST may act as a novel

multitarget therapy for MS combining its well-known immunomodulatory effect with a neuroprotective role based on stimulating remyelination.

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T02-010B

Sibling Cell Relationships of cell Progeny of Single Subpallial Progenitor Cells Using the UbC-StarTrack

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Over the last decades a growing number of evidences pointed out on the heterogeneity of neural progenitor cells (NPCs). Neural development requires a perfect balance between proliferation and differentiation of NPCs to give rise a functional adult nervous system. In our research, we are focused on the heterogeneity of NPCs, to unravel the diversity of the different neural lineages. NPCs give rise to distinct neural cell types at different embryonic and adult stages increasing the cell diversity in the brain. Using a multi-color genetic lineage tracing system, *UbC-StarTrack*, we targeted specific progenitor cells to analyze the cell type and location of their adult cell-derived progeny as well as their lineage relationships. To this end, we designed different StarTrack strategies to permanently label the individual progenitor cells and their progeny accordingly with the identity of the NPCs (Sánchez-Gonzalez et al 2020a). At this regard, we performed a new PiggyBac transposase under the expression of Gsh-2 promoter (called *UbC-(Gsh-2-hyPB)-StarTrack*) to specifically target progenitor cells located in the ventral areas of the ventricular surface of early mouse embryo. The derived-cell progeny of those subpallial progenitors produce different neural types (astrocytes, oligondrocytes, NG2 and even neurons), displaying diverse clonal patterns in relation to both their ontogenic origin and cell identity.

Our findings provide fundamental aspects of the progenitor potential, cell fate and the clonal relationships of their derived-cell progeny that might help to gain new insights into their behavior, complexity and functionality.

Acknowledgement

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T02-011B

Direct conversion of human fibroblasts to astrocytes

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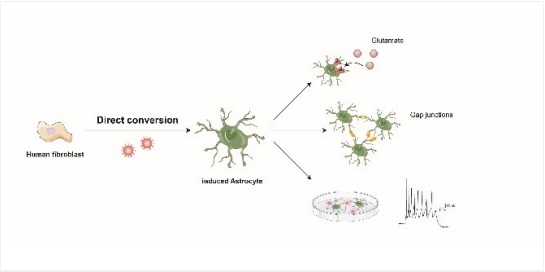
Astrocytes are essential cells in normal brain function and are emerging as key players in neurological disease. Traditionally, studies have been performed using rodent astrocytes but these are considerably less complex compared to their human counterpart. Over the recent years we and o--thers have shown that it is possible to generate astrocytes from human pluripotent stem cells (hPSC). These hPSC-derived astrocytes have been used to model several aspects of genetic neurological disorders and provided insight in disease mechanisms. However, during reprogramming to pluripotency the epigenetic landscape of donor cells is reset leaving the iPSCs at an embryonic like state. Thus, when modeling sporadic and late-onset diseases potential important information might not be revealed due to the cells inability to properly mimic the age of a patient or epigenetic components to disease.

Studies of neurons obtained through direct conversion of fibroblasts, in which the donor cells do not go through a pluripotency stage, have shown that the cellular age is preserved. However, only few studies have explored the generation of human astrocytes through direct conversion and efficiency as well as characterization of the obtained cells remains insufficient.

Here we describe efficient direct conversion of human embryonic fibroblasts to functional induced astrocytes (HEF-iAs). Through lentiviral-mediated overexpression of the transcription factors Sox9, Nfia and Nfib we obtain HEF-iAs that have a morphology that resemble primary human astrocytes, express astrocytic proteins and have a gene expression profile similar to that of primary human fetal astrocytes. Furthermore, HEF-iAs has the capacity to take up glutamate and form functional gap junctions, key functions of astrocytes. Interestingly, optimisation of the conversion conditions significantly increased yield of HEF-iAs. Importantly, this also improved the conversion of human adult fibroblasts to astrocytes, a necessity to enable disease modelling of age-related neurological diseases. Furthermore, we can for the first time show electrophysiological analysis of a co-culture system of neurons and astrocytes obtained through direct conversion from the same starting fibroblast population.

Direct conversion of human fibroblasts to astrocytes have the potential to add a higher dimension of complexity to current in vitro human astrocyte models and become useful for modelling late-onset neurological disorders.



Gliogenic transcription factors convert human fibroblasts to functional induced astrocytes
We have optimized a method to generate induced astrocytes through direct conversion of human fibroblasts by ectopically express gliogenic transcription factors and apply astrocyte promoting molecules. These cells display astrocytic properties and functions. We also show for the first time a co-culture setting of neurons and astrocytes obtained through direct conversion.

T02-012B

Coordinated activities between the Hedgehog and androgen signaling pathways during perinatal oligodendrogenesis

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During development, Hedgehog morphogens orchestrate morphogenesis by controlling cell growth, differentiation and migration (Briscoe & Small, 2015). The canonical signaling occurs when Hedgehog proteins bind the 12-pass transmembrane protein Patched (Ptc), which relieves the repressive activity exerted by Ptc on the seven-transmembrane protein Smoothened (Smo) then leading to a complex downstream signaling cascade involving the transcription factors of the Gli family (Kong, Siebold, & Rohatgi, 2019).The pathwayhas been previously involved in the perinatal wave of oligodendrocyte production arising from the germinative zone of the dorsal forebrain. Hedgehog signaling is known to act in a concerted manner with several other signaling pathways. However, the existence of possible coordinated effects with the androgen signaling pathway, which itself plays a key role in establishing the sexual phenotype of myelin in the developing brain (Abi Ghanem et al, 2017) has not yet been investigated. Here, we report stage-specific activities of Smo-mediated Hedgehog signaling and androgen receptor (AR)-mediated androgen signalling during early perinatal oligodendrogenesis in male animals. We show that the pathways induce distinct effects on neural stem cells (NSCs) giving rise to oligodendrocytes. Smo activation is required (as expected) for the maintenance of NSCs while AR signaling directs NSC differentiation toward the oligodendroglial lineage at the expense of astrocytes, thus revealing a yet unsuspected and nonreproductive function of AR in the early developing brain. Smo activation then promotes OPC proliferation but impedes the prominent pro-myelinating activity of testosterone detected both *in vitro* in glial cell cultures and *in vivo* in early postnatal mouse pups. The pro-myelinating role of AR does not require the aromatase-dependent conversion of testosterone into estradiol. This role is consistent with the increase in testosterone brain levels previously shown during the neonatal period in male animals and with the strong up-regulation of AR transcription starting from P8 and, which is strictly correlated with the up-regulation of the main myelin protein, MBP. We propose a model according which at the perinatal time, Smo-mediated Hedgehog signal is involved in the maintenance of NSCs whose fate determination is promoted toward the oligodendroglial lineage via AR-mediated signaling. OPC proliferation is then increased in a Smo-dependent manner before the down-regulation of the main transcriptional effector Gli-1 and the concomitant up-regulation of AR and MBP. The work provides evidence for a tight coordination between the Hedgehog and androgen activities during developmental myelination.

T02-013B

Physical Activity heterogeneously modulates NG2-glia population behavior, and is necessary for cognitive enhancement

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NG2-glia constitute about 2-10% of the total cell population in the CNS. They form oligodendrocytes (OLs), which generate myelin. Although most OLs and myelin rise shortly after birth, oligodendrogenesis still keeps going during adulthood -beyond the intrinsic myelination- in an experience-dependent fashion. The mechanism behind this remains still unclear, although strong evidence suggests that experience-dependent changes in neuronal activity regulate NG2-glia distribution, proliferation, and differentiation in the adult mouse brain. Thus, one possibility is that neuronal activity boosts NG2-glia differentiation and, in turn, newly generated OLs (ngOLs) promote myelination. Physical activity, a common form of experience, increases the *in vivo* motor cortex neuronal activity as well as the NG2-glia differentiation, but so far, the latter dynamics and functionality have been poorly analyzed. Therefore, in this study, we have used a model for voluntary physical activity (VPA), which consists of providing mice unlimited access to running wheels. Our results displayed an increase in the proliferation and differentiation of NG2-glia after VPA, a process, and region specificity to the grey matter of the motor cortex. Furthermore, we performed proteome analysis of sorted NG2-glia after VPA, and we found that they show less myelin and myelination-related proteins. Interestingly, proteome data correlated with an increase in the number of $GPR17^+$ NG2-glia, a subset of NG2-glia characterized by its slow differentiation rate. We tested specifically the differentiation behavior of $GPR17^+$ cells after VPA, and observed that despite VPA promotes their differentiation, this happens at a slower rate and in a lower magnitude. Finally, we aimed to answer the question of the functionality of ngOLs after VPA. It is known that VPA enhances cognitive performance in several tasks, such as learning and memory. This improvement nowadays has been exclusively related to increased neurogenesis. To understand the role of the ngOLs and therefore the new myelin formation after VPA, we prevented ngOLs formation through the $Sox10iCreER^{T2}xCAG\text{-}GFPxEsco2^{fl}$ mouse line, which leads to a block of NG2-glia differentiation. Thereby, we observed by a memory-related test -the novel object recognition test-, that the VPA-induced enhancement of cognitive performance was abolished. These novel results identify for the first time a role of adaptive myelination in the cognitive improvement that takes place after physical activity.

Acknowledgement
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T02-014B

Inducing fast-spiking neurons from glia in the postnatal cerebral cortex

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Direct lineage reprogramming of resident glia into induced neurons (iNs) is an emerging concept for the remodeling and restoration of diseased circuits. Using developmentally inspired transcription factors, glia-to-neuron conversion has been successfully achieved *in vivo* (Vignoles et al 2019).
Several neurological and neuropsychiatric disorders have a developmental origin. Thus, the early postnatal cortex may be the substrate of choice for remodeling circuits affected by neurodevelopmental disorders. Here, we aimed at testing whether the proneural transcription factor Ascl1 in combination with Bcl2 (Gascón et al 2016) can reprogram glia undergoing developmental expansion (i.e., via proliferation in the absence of prior injury) into functional iNs in the early postnatal cortex. For this, we transduced neonatal (P5) proliferating glia with retroviruses

encoding the reprogramming factors and explored the electrophysiological properties of these cells in acute brain slices.

We found that cells transduced with Ascl1 and Bcl2 acquired membrane properties similar to immature neurons, displaying transient inward currents and fired single action potentials. Intriguingly, forced co-expression of the phospho-deficient variant Ascl1SA6 (Ali et al., 2014) and Bcl2 resulted in the generation of iNs capable of repetitive action potential firing. At 4 weeks post injection, Ascl1SA6-Bcl2 derived iNs developed fast-spiking (FS) properties characterized by sustained high-frequency firing (>150 Hz) and received excitatory synaptic inputs. Consistent with these electrophysiological hallmarks of FS-interneurons, cells expressed Kv3.1 channels.Taken together, our data show the potential of a phospho-deficient mutant of Ascl1 to induce FS-interneuron specific features from glia *in vivo*.

Acknowledgement

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T02-015B

Reconstructing Enteric Nervous System lineages at single-cell resolution

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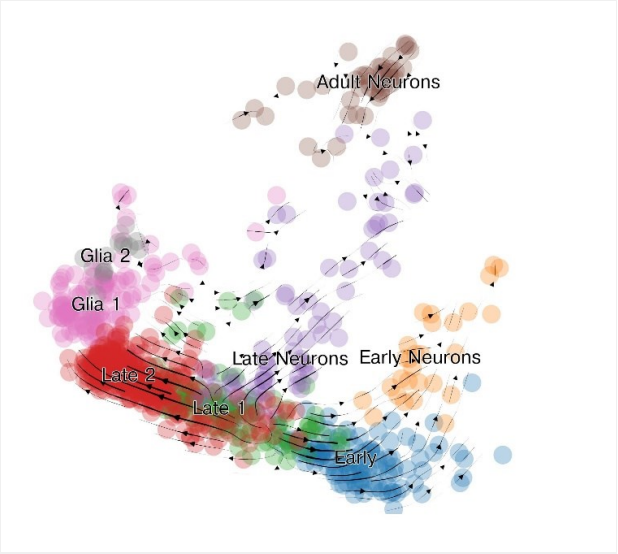
The enteric nervous system (ENS) plays an essential role in gut function and homeostasis, yet its development is not completely understood. Both enteric glial and neuronal lineages emerge from migratory neural-crest derived progenitors. Understanding the molecular machinery underlying the progression from precursor to neuron/glia, and factors underlying lineage commitment, could shed light on pathologies involving the ENS and may, in future, enable the design of targeted therapies.

To study ENS development we have collected single-cell RNA-seq data from murine Sox10-labelled FACS sorted ENS progenitor cells collected at multiple embryonic and post-natal time points, in addition to mature neurons and glia. Our results show that gliogenesis forms a default "linear" path of progenitor maturation, from which neurogenic trajectories branch off at early and late embryonic time points. However, despite differences in progenitor character, a core transcriptional program underlies neurogenesis at both time points. We identify a module of genes, upregulated in early progenitors, which we believe underlies neurogenic output. Around birth, ENS progenitors start expressing gene modules (enriched for immune processes, extracellular matrix organisation, response to lipid) which suggest an adaptive behaviour to the changing environment of the gastrointestinal tract. To facilitate our analysis of the geometry of single-cell pseudotime trajectories we have created an R Bioconductor package called TrajectoryGeometry. This allows one to ask whether changes in gene expression over pseudotime determine a well-defined direction, consistent throughout the course of a trajectory, or whether there are points where cells change course.

Although considered a terminally differentiated cell type, our data show mammalian enteric glial cells (EGCs) to be the end product of progenitor maturation. To test the possibility that glia can revert to progenitor-like state and can

undergo neurogenesis, we developed a robust experimental system that uncovers the latent neurogenic potential of EGCs. We find that enteric glia are able to give rise to neurons in culture, using "neural stem cell" and "differentiation" media, efficiently without the introduction of any reprogramming factors. Furthermore, time course bulk RNA-seq data show they upregulate genes characteristic of early ENS progenitors at DIV4, suggesting they retain progenitor-like plasticity. The expression of neurogenic genes, such as Ascl1 and Ret, was induced in these cells by DIV11 and was demonstrated to be required for glia-derived neurogenesis. Taken together, our results show that EGCs are capable of activating programs of gene expression that recapitulate key stages of neurogenesis during embryonic development.



Developmental trajectory
RNA velocity shows neurogenic trajectories emerging from ENS progenitors at both early (E12) and late (E16 and P00) embryonic/postnatal time points, whereas gliogenesis forms a default "linear" path of progenitor maturation.

T02-016B

Ultrastructural diversity of human oligodendrocytes and its progenitors in the white matter.

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Oligodendrocytes (OLs) are the myelinating cells of the central nervous system. They provide trophic, metabolic and structural support to neurons. In several pathologies such as multiple sclerosis these cells are severely affected and fail to remyelinate thereby leading to neuronal death. The gold standard for studying remyelination is

the g-ratio which is measured by transmission electron microscopy (TEM). Therefore, studying the fine structure of the OL population in the human brain at different stages through TEM is a key feature in this field of study. Here we analyze the ultrastructure of OLs, its progenitors and myelin in 10 samples of human white matter which were obtained from surgical resections from focal cortical dysplasia patients operated at Hospital Universitari i Politècnic La Fe at Valencia, Spain. Samples included in this study comprehend non-affected white matter of female and male patients (ages: 2-44 years-old). We used 9 different markers of OL lineage (NG2, PDGFRa, A2B5, Sox10, Olig2, BCAS1, APC (CC1), MAG and MBP). Our findings show that human OLs are a very heterogeneous population within the human white matter and that its stages of differentiation present characteristic features that can be used to identify them by TEM. Three defined ultrastructural populations were found. The first included NG2, A2B5, PDGFRa-positive large electron lucent cells with abundant intermediate filaments and short dilated endoplasmic reticulum. The second population comprised BCAS1 and Sox10-positive cells, which were characterized as small electron dense cells with scarce cytoplasm and numerous expansions contacting newly-formed myelin. The third population described were APC-CC1-positive cells whose ultrastructural features were: dark electron dense cells with abundant short and dilated endoplasmic reticulum and condensed chromatin attached to the nuclear membrane. Interestingly, all of the populations were labelled for Olig2. This study could shed light on how these cells interact with other cells within the human brain and clarify their fine characteristics from other glial cell types.

T02-017C

The role of Aβ oligomers in the myelin regulatory factor MYRF regulation and oligodendrocyte differentiation

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Amyloid β (Aβ) oligomers have been proposed as a candidate to promote oligodendrocyte and myelin dysfunctions, events related to the onset of the neurodegeneration in Alzheimer's disease (AD). Oligodendrocytes are glial cells responsible for the synthesis and maintenance of myelin in the CNS. Their differentiation and myelination processes are tightly controlled by several transcription factors, among which the Myelin Regulatory Factor (MYRF) is of the utmost importance. MYRF expression is strongly induced during the early stages of oligodendroglial differentiation and the maintenance of myelin in adults. MYRF is an ER-associated protein that needs to undergo a self-cleavage to release its N-terminal fragment from the ER and enter the nucleus, where it works as a transcriptional factor inducing the expression of essential myelin related genes such as MBP, which has itself been documented to be upregulated in presence of Aβ. Nevertheless, although MYRF is not only important but also crucial for oligodendrocyte maturation and CNS myelination, little is known about the effect that Aβ has in this transcriptional factor or the role it may play in AD. Here, with the aim of investigating the effect of Aβ in the oligodendroglial linage, using EdU and immunostaining assays, we reported that the dynamic of the oligodendroglial population is altered in 3xTg-AD mice corpus callosum with increased numbers of proliferative and newly generated mature oligodendrocytes. Furthermore, we demonstrated that the intrahippocampal injection of Aβ

also induces a similar effect, with promoted cell differentiation and maturation in both corpus callosum and dentate gyrus of injected mice, suggesting that alterations observed in 3xTg-AD mice were at least in part driven by Aβ. In addition, levels of nuclear MYRF were also increased in injected mice oligodendrocytes. These finding agree with *in vitro* results showing that Aβ treatments enhance total and cleaved nuclear MYRF levels in cultured rat oligodendrocytes transfected with 2xtagged MYRF expression construct Myc-Myrf-Flag, suggesting an increase of Aβ-induced MYRF's cleavage and nuclear activity. Therefore, we finally propose using Transmembrane protein 98 (TMEM98), a protein that inhibits the self-cleavage and activity of MYRF, to normalize Aβ-induced alterations in oligodendrocytes and myelin related proteins. Results obtained by the forced expression of TMEM98 in rat oligodendrocytes showed reduced nuclear MYRF levels and MBP expression. These results together suggest that Aβ oligomers may act on oligodendrocyte maturation and differentiation by mechanisms implicating the activity of MYRF; and, thus, normalizing the increased MYRF's activity using TMEM98 might be a novel strategy to ameliorate Aβ-induced dysfunctions.

T02-018C

The Role and the Mechanism of Septin7 in Proliferation, Migration and Differentiation of Oligodendrocyte Precursor Cells

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The highly dynamic cytoskeleton of oligodendrocyte lineage is a main driver for the motile behaviors and morphological changes that are interrelated with oligodendrocyte precursor cells (OPCs) process outgrowth, primary membrane wraps and myelin formation. Septins belongs to a family of GTP-binding proteins, which is recognized as a novel component of the cytoskeleton and is believed to function as a significant element in some dynamic, functionally active cellular processes. Septin7(Sept7) is unique out of 13 septins which has been found expressed in the myelin sheath in central nervous system (CNS) and involved in myelin formation and remyelination in our previous studies. However, the role of Sept7 in OPCs proliferation, migration and differentiation and that underlying mechanisms are still unknown. In present study Immunofluorescence (IF), Q-PCR, and Western Blot (WB) were performed to analyze the expression of Sept7 in primary cultured OPCs and OLN-93 cells, as well as its spatial relationship with microfilaments and microtubules. After lentivirus-mediated small interfering RNA targeting Sept7 administration, the changes of proliferation, migration and differentiation of OLN-93 cells were detected by CCK8 assay, Scratch assay and morphological methods, respectively. And the expression of cytoskeleton and myelin proteins were measured by WB and IF. The results showed that Sept7 expression was correlated with the differentiation of OPCs, and its distribution was spatially closely interacted with F-actin and α-tubulin. After Sept7 was knocked down in OPCs, several morphological changes including loss of the polarity processes with short brush-like projections extending occurred accompanied with decreased the proliferation and increased migration. More critically, Sept7 knock down led to OPCs differentiate failure which lack of membranous processes and decreased expression of mature oligodendrocytes markers including MBP and CNP. Simultaneously, the spatial distribution and expression of F-actin and α-tubulin as well as acetylation level of α-tubulin were changed. These results suggest that Sept7 plays an important role in proliferation, migration and differentiation of OPCs through interacting with microfilaments and microtubes, and Sept7 might have regulatory effect on myelin proteins expression.

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T02-019C

Investigating the transcriptional changes underpinning enhanced oligodendrocyte maturation after exposure to microglia-derived vesicles

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Degeneration of myelinating oligodendrocytes (OLs) and consequent disruption of the myelin sheath enwrapping neuronal axons represent major contributing factors to neurodegeneration and disability in several neuropathological conditions. On this basis, fostering endogenous myelin repair sustained by oligodendrocyte precursor cells (OPCs) is considered a promising therapeutic approach to preserve neuronal integrity and to counteract disease progression [1]. A central role in shaping remyelination is played by microglia, that are able to regulate both myelin damage and repair processes by acquiring different functional states [2]. In this respect, extracellular vesicles (EVs) released by microglia recently emerged as pivotal players in their communication with OPCs. When administered *in vivo,* EVs derived from pro-regenerative microglia efficiently enhanced OPC maturation and remyelination of the demyelinated lesion caused by lysolecithin brain injection or experimental stroke [3,4]. Furthermore, results from *in vitro* experiments highlighted the capacity of microglial EVs to directly foster OPC proliferation, migration, differentiation and myelination of neurons in co-culture [3,4]. However, the mechanism underlying EV-induced beneficial effects on OPCs is still obscure. Here, we performed a transcriptomic profiling of primary OPCs exposed to microglial EVs to detect early molecular changes which may be responsible for enhanced OL maturation induced by EVs. Briefly, cultured microglia were stimulated with Th1 cytokines or IL-4, to trigger a pro-inflammatory or pro-regenerative activation respectively, or co-cultured with immunosuppressive mesenchymal stem cells (MSCs) in the presence of Th1 cytokines, a condition also driving pro-regenerative traits in microglia [3]. Then, EVs derived from pro-inflammatory (i-EVs), pro-regenerative (IL-4 EVs) microglia, and cells co-cultured with MSCs (MSC-EVs) were collected by differential centrifugation and added to primary OPCs for 24 hours during their differentiation. Transcriptomic analysis of OPCs receiving EVs or vehicle (CTRL) was carried out using Clariom™ D mouse arrays, revealing prominent changes induced by protective types of EVs compared to CTRL (3700 DEGs MSC-EVs vs CTRL; 1143 DEGs IL-4 EVs vs CTRL), while i-EVs were less potent (103 DEGs i-EVs vs CTRL). Ingenuity Pathway Analysis (IPA) software has been exploited to identify the molecular pathways significantly modulated by EVs, showing that most of them were found to be in common between IL-4 EVs and MSC-EVs, and to predict the upstream regulators that might be responsible for these transcriptional changes. The validation of these preliminary findings will help to define novel molecular pathways to be exploited for remyelinating therapies.

Acknowledgement

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T02-021C

Effect of two Multiple sclerosis biomarkers, chitinase 3-like protein 1 and syndecan-1, on oligodendrocyte precursor cells proliferation, migration and differentiation

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Background: Multiple sclerosis (MS) is an autoimmune and neurodegenerative pathology characterised by demyelinated plaques in the CNS. Remyelination is an active repair process involving oligodendrocyte precursor cells (OPCs) proliferation and differentiation. It is positively correlated with slower progression of MS and a prolonged survival of MS patients. With time, tissue remodelling becomes less and less effective, leading to a glial scar and to neurodegeneration. Previously, we identified an increased Chitinase 3-like protein1 (CHI3L1) and Syndecan-1 (SDC1) expression in cerebrospinal fluid (CSF) as prognostic biomarkers of MS. CHI3L1 is a secreted protein involved in extracellular matrix remodelling and commonly expressed during inflammation. On the other hand, SDC1 is a widely expressed transmembrane protein involved in cell-matrix interactions. In other pathologies such as cancer, CHI3L1 and SDC1 interaction has already been described to play a role in proliferation, adhesion, migration and differentiation processes. Finally, in human brain, we observed an important expression of CHI3L1 by astrocytes and of SDC1 by glial cells within MS plaques. However, the role of CHI3L1 and SDC1 in demyelination and remyelination is largely unknown. Here, we aim to analyse the influence of CHI3L1 and SDC1 possible interaction on proliferation, differentiation and migration of OPCs.
Methods: First, we exposed primary cell cultures of rat OPCs to recombinant human CHI3L1 (rhCHI3L1) to explore CHI3L1 impact on OPCs processes by microscopy. We monitored OPCs proliferation by EDU labelling, OPCs differentiation using maturation markers (NG2, O4) by immunolabelling and OPCs migration by agarose drop assay. We analysed SDC1 timing expression during OPCs differentiation by immunolabelling.
Results: In primary cell culture, we did not observed any impact of rhCHI3L1 on OPCs proliferation., Cell migration was slightly increased by exposure to rhCHI3L1. We observed an increase of NG2+ cells and a decrease of O4+ cells in rhCHI3L1 condition, suggesting that rhCHI3L1 had a transient impact on OPCs differentiation *in vitro*. Moreover, O4+ cells expressed the majority of SDC1 in OPCs primary cell culture.
Discussion: OPCs migration occurring during early stages of differentiation, the increase observed could be a direct effect of CHI3L1 or a side effect of the differentiation slow down effect of CHI3L1. We also demonstrate a strong SDC1 expression in early OPC stages of differentiation. The timing of rhCHI3L1 effects and SDC1 expression support a possible interaction between these two MS biomarkers in OPCs. Next step will be to study the CHI3L1 and SDC1 interaction in OPCs at early stages of differentiation and the pathway(s) involved.

T02-022C

Fast and efficient generation of oligodendrocytes from human induced pluripotent stem cells (hiPSCs)

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Background:
Oligodendrocytes (OLs) are highly specialized cells of the central nervous system (CNS) responsible for myelin production and metabolic support of neurons. Defects in OLs are crucial in several neurodegenerative diseases including multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Scarce access to primary samples and lack of efficient protocols to generate OLs from human pluripotent stem cells (hPSCs) are hampering our understanding of OL biology and the development of novel therapies.

Methods:
To promote the conversion of hPSCs into OLs, we have screened for a number of transcription factors (TFs) previously reported to be involved in OL generation. For this, hPSCs were fated for 8 days toward neural progenitors, and then transduced with an inducible lentiviral vector encoding for the different TFs.

Results:
We found that the overexpression of SOX10 was sufficient to generate O4+ oligodendrocyte precursor cells (OPCs) from hPSCs only 10 days after SOX10 induction. Generated OPCs expressed mature OL proteins as MBP or MOG. At the transcriptome level, generated OPCs resembled primary OPCs. To date, OPCs have been derived from eight different hPSC lines including those derived from patients with spontaneous and familial forms of MS and ALS, respectively. To test the functionality of generated OPCs, O4+ cells were co-cultured together with hPSC-derived neurons for additional 20 days, finding that O4+ cells were able to myelinate the neurons. Moreover, O4+ cells were injected intracerebrally in newborn shiverer RAG2$^{-/-}$ mice and the tissue was examined 16 weeks later, finding that generated OLs extended within the corpus callosum and generated functional myelin, demonstrating the functionality of generated cells also in vivo. The protocol also describes an alternative for viral transduction, by incorporating an inducible SOX10 in the safe harbor locus AAVS1, yielding ~100% pure OPCs. O4+ OPCs can be co-cultured with maturing hPSC-derived neurons in 96/384-well- format plates, allowing the screening of pro-myelinating compounds.

Conclusions:
We have developed a novel methodology for a fast (20 days from hPSC stage) and efficient generation of functional OLs, which allow testing of compounds involved in myelination. This technology will allow further studies to better understand human OL biology and the screening of potential compounds involved in myelination in a human setting.

Acknowledgement
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T02-023C

Characterization of the molecular mechanisms of Tns3 function in oligodendroglia

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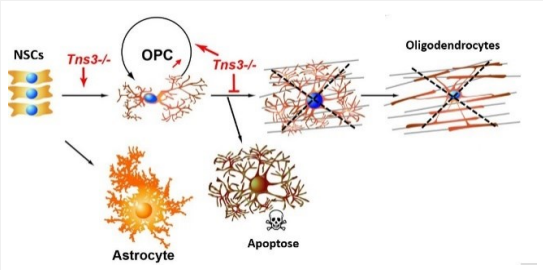
Multiple sclerosis (MS) is a neurological disease characterized by a loss of oligodendrocytes, the myelinating cells of the Central Nervous System. Despite recent advances leading to stop the immune system from attacking oligodendrocytes, efficient remyelinating therapies are still lacking. In MS, the spontaneous remyelination from the oligodendrocyte precursor cells (OPCs) present all over the brain is inefficient and diminishes with age. The observation that OPCs are present within demyelinating MS lesions, but fail to differentiate into myelinating cells, suggests that induction of OPC differentiation is a critical event for successful remyelination. We have recently identified *Tns3* (*Tensin 3*) as a gene target of Ascl1 and Olig2, two key oligodendrogenic transcription factors, and showed that *Tns3* is strongly induced at the timing of oligodendrocyte differentiation while downregulated in mature oligodendrocytes, constituting a good marker for immature oligodendrocytes.

Using $Tns3^{Tns3\text{-}V5}$knock-in mice, we found that Tns3 protein tagged at C-terminal end with the small V5 epitope was detected specifically in oligodendroglia, where it is mainly restricted to immature OL stage, being localized to the perinuclear cytoplasm and cell processes. This expression pattern is the also found during adult brain remyelination after LPC injection, where Tns3 is found in newly formed OLs. Therefore, Tns3 constitutes a novel marker for immature OLs.

In vivo *Tns3* loss-of-function (LOF) by CRISPR/Cas9 technology in neonatal neural stem cells (NSCs) of the subventricular zone blocks oligodendrocyte differentiation without affecting OPC survival or proliferation. We reproduced this differentiation defect in OPC differentiating cultures using an AAV9 vectors to induce CRISPR-mediated *Tns3* LOF.

We generated a $Tns3^{Flox}$ mouse allele, and are currently inducing an OPC-specific *Tns3* LOF in vivo and in vitro to confirm these results and explore cellular phenotypes of mutant OPCs. Using the V5-tagged *Tns3* mouse line, we will perform proteomics to identify Tns3 partner proteins and understand its molecular mechanisms of Tns3 function in oligodendrocyte differentiation.



Tns3 KO impair oligodendrocyte differentiation
CRISPR-Cas9 *Tns3 in vivo* Knock Out impairs mature oligodendrocyte generation without affecting OPCs proliferation or survival.

T02-024C

Transcription regulation of gliogenesis: a play between transcription factors and chromatin remodelers

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Oligodendrocytes (OLs) are myelin-forming cells of the central nervous system wrapping axons and allowing the saltatory conduction of action potentials. These cells arise from the differentiation of oligodendrocyte precursor cells (OPCs), which requires significant genetic reprogramming implicating transcription factors but also chromatin remodelers. We have previously demonstrated the role of two of those, Chd7 and Chd8, in some aspects of OPC biology, like differentiation, proliferation and survival. Both of them are involved in diseases affecting brain development as mutations in Chd7 and Chd8 gene cause CHARGE syndrome and Autism Spectrum Disorder (ASD), respectively. In this study, we used OPCs as a model to understand the specificity of Chd7 and Chd8, as well as their compensatory effect on each other. To that purpose, we are looking at OPC processes and transcription deregulation (RNA-seq) caused by the loss of each factor or both. With the help of binding profiles (ChIP-seq) of these factors, we aim to understand their direct regulation in OPC biology. This study will allow a better understanding of the molecular mechanisms controlled by two chromatin remodelers involved in brain development and diseases.

T02-025D

Pharmacogenomic identification of key genes and small bioactive molecules promoting oligodendrogenesis in the model of neonatal brain injury

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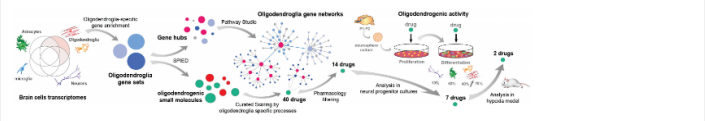
Preterm birth (PTB) is the commonest cause of death and disability in children under 5 years, affecting 15 million infants yearly born before 37 gestational weeks. Rates are increasing in developing countries (e.g. 7% in France and UK, 13% in the US), associated with a high level of morbidity and neonatal encephalopathy leading to persistent cognitive and neuropsychiatric deficits (such as autism spectrum, attention-deficit disorders and epilepsy). Two key processes involved are alterations of oxygen concentrations and perinatal neuroinflammation that dramatically affects survival and maturation of oligodendrocytes precursor cells (OPCs). As a consequence, PTB injury manifests as hypomyelination, interneuron deficit, abnormal connectivity, and synaptopathy. Indeed, in both humans and rodents, the perinatal period, is a time of active oligodendrogenesis, myelination, and axonal growth within the developing subcortical white matter. Because of the long-term consequences and the absence of current treatment, there is an urgent need to develop novel therapeutic approaches for promoting brain repair through oligodendrocyte (OL) regeneration. Thus, we established a computational-based drug-discovery strategy in order to control specifically germinal activity by newly specific identified small bioactive molecules. As a matter of fact, genetic lineage tracing experiments probed that neural stem cells (NSCs) from the dorsal subventricular zone are the major source of postnatal OPCs, also keeping their capacity to contribute to adult brain remyelination. Interestingly, the main part of maturational brain growth, equivalent to the human brain in the last trimester of pregnancy, occurs during the first postnatal week in rodents, allowing easier experimental manipulations of the postnatal SVZ. Based on genome-wide gene expression datasets levering the power of mouse genetic tools, we produced gene sets enriched in oligodendroglia and identified key transcriptional networks and regulators of postnatal oligodendrogenesis (i.e. specification, proliferation, survival, and/or differentiation of OPCs). Then, using both enriched gene sets generated and a knowledge-driven curation gene sets, we performed a pharmacogenomics analysis to identify set of small bioactive molecules that could potentially foster oligodendrogenesis. Using neonatal NSC differentiation cultures, we validated their capacity to strongly promote oligodendrogenesis. We further selected and defined optimal posology of the most promising molecules in order to test their capacity in vivo using both postnatal hypoxia and adult de/remyelination mouse model (ongoing). Overall, this project could provide new innovative therapies for preterm infants and demyelinating diseases such as multiple sclerosis.



Identification of small bioactive molecules and regulatory networks promoting oligodendrogenesis
Strategy to generate gene sets, networks and hubs enriched in oligodendroglia, followed by a pharmacogenomic identification of small bioactive molecules. Drug candidates were then sorted using their target genes and a scoring procedure involving curation of all processes involved in oligodendrogenesis. Drugs were filtered for their phamacodynamic properties for validation of their oligodendrogenic capacity in neonatal NSCs cultures. Among 7 drugs showing higher pro-oligodendrogenic capacities *in vitro*, we selected 2 top drugs for further study using an *in vivo* hypoxia model.

T02-027D

Stability of lineage-specific attributes in senescent human peripheral glia

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Senescent and non-senescent human Schwann cells (hSCs) established in culture are virtually undistinguishable without the aid of specific tests such as detection of senescence-associated β-galactosidase (SA-βGal) activity. In most cultures, the rate of cell division is maintained high up until the second passage but cells cease to proliferate rapidly thereafter and the populations become senescent. By passage-5 no further expansion is possible and the cells manifest abnormalities such as vacuolization of the cytoplasm, aberrant nuclei (including multinucleation), and clustering. Arrival to senescence in hSC populations cannot be prevented by overexpression of hTERT and indefectibly occur under standard culture conditions regardless of the age of the donor. Even though senescent hSCs remain viable for prolonged periods of time, it is unclear whether they maintain attributes specific to cells of the SC lineage. To address this question, we performed a careful analysis of the progression of hSCs towards senescence to evaluate changes in proliferation rates, viability, purity and expression of SC-specific markers. We also obtained the transcriptomes of hSCs collected at different rounds of subculture and performed a stringent bioinformatics analysis to identify SC-specific and regulatory genes. We found that the hSC transcriptome was very stable and that hSCs maintained their expected identity (or transcriptional signature) regardless of subculture and the continued influence of mitogenic factors. Strikingly, the transcriptomes of low passage (proliferative) and late passage (senescent) hSCs were essentially undistinguishable with the exception of <100 differentially expressed genes known to play a role in replicative senescence, cell cycle arrest, chromatin organization and telomere maintenance. Senescent hSCs expressed invariable levels of SC-specific markers such as S100β and aligned to each other forming typical bundles at confluency. Most importantly, they maintained their ability to engulf and digest myelin granules, which is a function proper of SCs during nerve repair. To conclude, our studies show the value of combining transcriptomics (RNAseq) profiling and cell-based assays to understand hSC senescence. The stability of the hSC transcriptome in the face of expansion and mitogenic stimulation adds a level of safety for the use of these glial cells in autotransplantation therapy.

Acknowledgement

This work was supported by The Craig H. Neilsen Foundation (grant 339576 to P.V.M. and G.W) and the Indiana Traumatic Spinal Cord and Brain Injury Research Fund from the Indiana State Department of Health (to P.V.M and X.M.X).

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T02-028D

Pro-inflammatory IL-1β signalling regulates oligodendrocyte progenitor cells and microglia *in vitro* and *in vivo*

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Oligodendrocytes (OL) are the myelinating cells of the central nervous system (CNS). During development, oligodendrocyte progenitor cells (OPCs) are recruited to axons, proliferate and differentiate into mature OL that form new myelin sheaths. In multiple sclerosis (MS) the myelin sheath is damaged, however, myelin regeneration (remyelination) can occur in early stages of the disease but fails with disease progression, in part due to impaired OL differentiation. To date there are no treatments available that can therapeutically enhance remyelination.

Intriguingly, remyelination is boosted in areas of inflammation, while the absence of an inflammatory response impaired CNS repair. IL-1β a potent pro-inflammatory mediator was found in MS lesions but conversely, was also shown to enhance CNS repair in an animal model of MS. Its function in OPC biology, myelination and remyelination remains incompletely understood.

This project aims to determine the function of pro-inflammatory IL-1β signalling on glial cells in homeostasis and after damage.

Mixed glial and pure glial cultures were used to investigate the impact of IL-1 signalling on OPC biology and myelin protein production in context of other glial cells and in isolation. Upon exposure to IL-1 glial cells were fluorescently stained and survival, proliferation, differentiation and myelin protein production was quantitatively analysed.

IL-1β exposure enhanced differentiation of OPC and increased myelin basic protein (MBP) production, one of the major proteins of CNS myelin. On the other hand, IL-1β did neither affect glial cell survival, nor OPC proliferation. Enhanced OPC differentiation was mediated through IL-1R1 signalling in glial cells.

In order to investigate the role of IL-1 signalling on glial cells after myelin damage mice deficient of IL-1R1 were subjected to focal demyelination. OPC proliferation, differentiation as well as composition of glial cell populations within the lesion was quantitively assessed. Absence of IL-1R1 signalling did not affect OPC differentiation after damage but enhanced numbers of proliferative OPC early after demyelination. Microglial/macrophage populations were decreased, while astroglia were increased within lesions in mice deficient of IL-1 signalling.

We here show that depending on homeostasis or in injury context OPC differentially respond to IL-1□. Furthermore, IL-1R1 deficiency in an injury context enhanced astroglial but decreased microglial cell populations. As IL-1□ was found in MS lesions, this could be important for the outcome of remyelination. Further analysis will uncover the underlying mechanisms, which might prove valuable for the development of novel MS therapies targeting IL-1 signalling.

T02-029D

C21orf91´s role in oligodendroglial precursor cell differentiation and CNS myelination: a potential role for Down syndrome neuropathology

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Impaired differentiation of oligodendroglial cells and subsequent alterations in white matter structure and dynamics are common features of neurological diseases of the central nervous system (CNS). Down syndrome (DS), or trisomy 21, is the most prevalent aneuploidy and most common genetic cause for cognitive impairments and intellectual disability (ID). Neuropathological alterations in DS are characterised by a reduction in the number of neurons and an unusual, aberrant glial composition, resulting in hypomyelination and partial astrogliosis. Recent studies mainly focused on neuronal development in DS and underestimated the role of glial cells as pathogenic players. This also relates to C21orf91, a rarely described protein considered a key modulator of aberrant CNS development in DS. We examined the role of C21orf91 in terms of oligodendrogenesis and myelination using bioinformatics data evaluation tools and cultured primary oligodendroglial precursor cells (OPCs). Our gene modulation studies revealed that the C21orf91 ortholog gene is important for accurate oligodendroglial differentiation, influencing their capacity to mature and to myelinate axons. Interestingly, C21orf91 ortholog overexpression initiates a misguided cell fate resulting in a cell population co-expressing a number of astroglial- and oligodendroglial markers such as for example glial fibrillary acid protein (GFAP) together with myelin basic protein (MBP). This aberrant phenotype was also observed in overexpressing cells upon transplantation onto myelinating cocultures. Our observations therefore indicate that elevated C21orf91 expression levels induce a gliogenic shift towards the astrocytic lineage reflecting non-equilibrated glial cell populations in DS brains. Current investigations aim at clarifying C21orf91's role in gliogenesis by means of modulating neural stem cells (NSCs), a further source of CNS myelinating glial cells.

Acknowledgement

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T02-030D

Rebound effects of NCX3 pharmacological inhibition in oligodendrocytes

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The Na^+/Ca^{2+} exchanger NCX3 is an important regulator of sodium and calcium homeostasis in oligodendrocyte

lineage[1,2]. To date, no informations are available on the effects resulting from prolonged exposure to NCX3 blockers and subsequent drug washout in oligodendroglia. Here, we investigated the effects of the NCX3 inhibitor, the 5- amino- N- butyl- 2- (4- ethoxyphenoxy)-benzamide hydrochloride (BED)[3], on NCX3 expression and activity, as well as intracellular $[Na^+]_i$ and $[Ca^{2+}]_i$ levels, during treatment and following drug washout both in human MO3.13 oligodendrocytes and rat primary oligodendrocyte precursor cells (OPCs).BED exposure antagonized NCX activity, induced OPCs proliferation and $[Na^+]_i$ accumulation. By contrast, BED washout after 4 days of treatment significantly upregulated NCX3 proteins, reversed NCX activity, and increased intracellular $[Ca^{2+}]_i$., This BED effect was accompanied by an upregulation of NCX3 expression in oligodendrocyte processes and accelerated expression of myelin markers in rat primary oligodendrocytes. Collectively, our findings showed that the pharmacological inhibition of the NCX3 exchanger with BED blocker is followed by a *rebound* increase in NCX3 expression and reversal activity that may accelerate myelin sheet formation in oligodendrocytes. In addition, they indicate that particular attention should be paid to the use of NCX inhibitors for possible *rebound* effects, and suggest that further studies will be necessary to investigate whether selective pharmacological modulation of NCX3 exchanger may be exploited to benefit demyelination and remyelination in demyelinating diseases.

Acknowledgement

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T02-031D

Genetic approach to study the role of Sonic Hedgehog in physiological CNS myelination and remyelination.

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Sonic Hedgehog (Shh) is the best-studied ligand of the hedgehog signaling pathway. It is implicated in the regulation of vertebrate organogenesis, and in CNS, it plays a key role in the organization of the brain. Shh is

classified as morphogen forming a concentration gradient that has different effects on the developing embryo cells. Shh gradient continues to act in the adult brain, modulating self-renewal and specification of neural stem cells. Furthermore, hedgehog signaling is often upregulated after acute brain injury. Recent studies showed that progenitors in the postnatal V-SVZ of the dorsal forebrain require Shh signaling to generate oligodendrocytes in the corpus callosum. Oligodendrocytes are glial cells that myelinate axons and play critical roles in the development and function of neocortical circuits. There is a substantial population of oligodendrocyte precursor cells (OPCs) that remain during adulthood, required to support myelin plasticity and maintenance, as well as after episodes of demyelination as the one that occurs during Multiple Sclerosis. Shh has been shown over-expressed in Multiple Sclerosis lesions, and this pathway has the potential to regulate OPC responses that impact remyelination. However, the direct effect of Shh pathway on OPCs during postnatal development and in pathological conditions is still an open question. The current work shows how the up or down-regulation of Shh pathway affects OPCs differentiation both during postnatal and adult (re)myelination. Furthermore, through the transplantation of OPCs in areas of the CNS that do not present myelin under physiological conditions, such as the retina, it is shown that the effect of the activation/inhibition of this cascade is dependent on the environmental factors that differ depending on the stage of developing.

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T03 | Cell signalling

T03-001A

Probing the localization of the endoplasmic reticulum in the gliapil and its effect on astrocytic calcium signals

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Astrocytes respond to neuronal stimuli with calcium signals that can trigger the modulation of neuronal activity [1]. Most of those signals form so-called calcium microdomains[2], heterogeneously distributed within the cell, which could coincide with particular spatial arrangements of intracellular calcium stores. As the endoplasmic reticulum is responsible for the majority of calcium signals in astrocytes[3], its morphology could influence local calcium activity. However, as most signals occur in branchlets with a diameter < 200 nm, the associated ER morphology remains poorly characterized. Here, we use electron microscopy and reaction-diffusion simulations in realistic 3D geometries of astrocyte branchlets to investigate the causal relationship between the morphology of the ER and the spatio-temporal characteristics of ER-dependent calcium signals in fine astrocyte branchlets. Contrary to previous reports[4], we detect ER in fine branchlets, that can be less than 100 nm to the closest postsynaptic density. Simulations in simplified astrocyte branchlet geometries outline the effect of the morphology of the ER and of the distribution of calcium channels on calcium signals. Strikingly, predictions from simulations in simplified geometries no longer hold in more complex geometries extracted from electron microscopy. Our results suggest that ER morphology is important to regulate calcium peak frequency. This work, by combining computational and experimental approaches, provides new insights on the mechanisms that shape astrocyte calcium activity at the nanoscale.

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T03-002A

Cholesterol-induced calcium oscillation inhibits cellular death and supports lipid droplet formation in astrocytes

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Cholesterol is an abundant molecule, composing the plasma membrane of all cells. The brain is one of the most cholesterol-rich organs, containing ~20% cholesterol of the whole body. In several neurodegenerative diseases, metabolic abnormalities in cholesterol have been reported. Modulation of cholesterol biosynthesis improves the pathophysiology of Alzheimer's disease and Huntington's disease. And it was indicated that demyelination causes extracellular free cholesterol accumulation, reflecting that half of the myelin consists of cholesterol. However, the effects of the increase in extracellular free cholesterol in the central nervous system have been less focused, which was investigated in this study using calcium imaging. We discovered that bath application of cholesterol induced robust calcium oscillation in hippocampal primary culture, which was not affected by a Na channel blocker, tetrodotoxin. Immunostaining revealed that not neurons but astrocytes were responsible for the calcium oscillation. The cholesterol-induced calcium oscillation took place with a several minutes lag to the cholesterol application and was not terminated by washout. Cholesterol accumulation in the astrocytic plasma membrane was revealed by filipin dye staining, of which time course had a similar lag to the calcium oscillation. These results suggest that not the extracellular cholesterol per se but the increase of cholesterol concentration in the astrocytic plasma membrane triggered the calcium oscillation. Pharmacological experiments revealed that the calcium oscillation requires activation of phospholipase C (PLC) and inositol trisphosphate (IP_3) receptor, and depletion of extracellular calcium did not affect the calcium oscillation generation, indicating that the cholesterol-induced calcium oscillation requires the PLC-IP_3 pathway. Cholesterol treatment for 24 hr led to cell death in neuron-glia mixed culture, however astrocytic pure culture survived under high extracellular cholesterol situation at least for 48 hr. Inhibition of the calcium oscillation by 2-APB, an IP_3 receptor antagonist, led to cell death with less lipid droplet formation. In this study, we revealed that cholesterol induces robust calcium oscillation in not neurons but astrocytes, which may play a role in astrocytic survival under high-cholesterol situations under pathological conditions.

T03-003A

Neural S1P-lyase deficiency causes astrogliosis and affects energy metabolism

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Sphingosine 1- phosphate (S1P) is an evolutionarily conserved catabolic intermediate of sphingolipid metabolism that regulates diverse biological processes in the brain, including neural development, differentiation, and survival. S1P-lyase (SGPL1) irreversibly cleaves S1P in the final step of sphingolipid catabolism. To investigate the function of S1P in the brain, we generated a mouse model in which SGPL1 was explicitly inactivated in neural cells. Consequently, S1P accumulated in the brain causing, cognitive deficits and disruption of presynaptic architecture and function. The ablation of SGPL1 caused a block in neuronal autophagy that led to the accumulation of aggregate prone proteins like amyloid precursor protein. We now show that SGPL1 deficiency triggers astrogliosis. Thus the expression of GFAP was significantly increased in SGPL1-deficient murine brains as well as in astrocyte cultures derived thereof. Hence, an increased expression of inflammatory cytokines, including IL-6, IL-11, and TNFα, was detected in SGPL1-deficient astrocytes. Similarly, the expression of P2Y1 purinoreceptor (P2Y1R), another astrogliosis marker, was elevated in SGPL1-deficient murine brains as well as in cultured astrocytes derived thereof. This prompted us to quantify the amount of purine nucleotides. We found that the ADP/ATP ratio was higher in the extracellular medium of SGPL1-deficient astrocytes compared to controls, whereas intracellularly, the opposite was true. This can be explained by the observed S1PR2/4-mediated increase of the glycolytic flux in SGPL1-deficient astrocytes. Overall, our results indicate that energy metabolism and inflammation are linked via an S1P/S1PR mediated mechanism in SGPL1-deficient astrocytes.

T03-004A

Noradrenergic cAMP signaling in astrocytes of the murine olfactory bulb

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Astrocytes respond to a variety of neurotransmitters and modulators by activation of receptors and transporters. Most of those signaling cascades have been extensively studied in means of calcium signaling. However, many astrocytic receptors are coupled to G_i or G_s and initiate an intracellular rise or fall of cyclic adenosine 3´,5´-monophosphate (cAMP) concentration. cAMP is a ubiquitous second messenger and plays a central role in gene expression and synaptic transmission. While calcium imaging is a well-established method with a variety of different chemical and genetically encoded sensors, cAMP sensors have been lacking until recently and hence cAMP signaling is not well studied in astrocytes yet. Flamindo2 from Odaka et al. (PLOS One 9:6, 2014) is a genetically encoded cAMP sensor, which fluorescence intensity decreases in response to increased cAMP levels. In this study we investigated cAMP signaling in astrocytes of the main olfactory bulb by confocal cAMP imaging using Flamindo2 in acute brain slices. Therefore, we performed a retroorbital injection of the AAV-packed Flamindo2 construct controlled by the astrocyte-specific promotor GFAP. The olfactory bulb is innervated by noradrenergic projections from the locus coeruleus as main source of norepinephrine (NE). Those noradrenergic terminals are found in the glomerular layer and modulate neuronal plasticity and thus olfactory processing. Astrocytes express different NE-receptors; while α1-receptor-mediated calcium transients in olfactory bulb astrocytes have been shown, we asked whether NE also evokes cAMP signaling. Our results show that the application of NE results in changes in cAMP levels in astrocytes via different G-protein-coupled receptors. Bath application of 10 µM NE (30 s) induced transient increases in cAMP concentration. Suppression of neuronal activity by use of a mix of glutamatergic and GABAergic antagonists as well as tetrodotoxin did not affect NE-induced

cAMP signals. The application of the β receptor agonist isoprenaline evoked large cAMP signals, while α1 andα2 agonists phenylephrine and xylazine evoked small but measurable cAMP signals. NE-induced cAMP signals could be significantly reduced with a combination of antagonists prazosin (α1), rauwolscine (α2) and ICI 118,551 (β). Our results show that NE evokes cAMP signaling in olfactory bulb astrocytes mainly by activation of b receptors and to a lesser extent by α1 and α2 receptors.

T03-005A

Mechanotransduction in retinal glia

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Glaucoma patients may continue to lose visual function long after therapeutic stabilization of intraocular pressure (IOP) due to proinflammatory activation of retinal glia. Thus, o*nce triggered by mechanical stress, inflammation may become independent of it to drive visual field loss even in the presence of adequate pressure control*. It is not known whether retinal glial cells are intrinsically sensitive to mechanical stressors or their activation principally reflects the response to neuronal degeneration. To address this, we investigated mechanotransduction mechanisms in retinal Müller cells, microglia and astrocytes from healthy and glaucomatous mice.

Intraocular pressure (IOP) in mice was elevated with microbead injections. Transcript levels were assessed with semi-quantitative PCR, and protein expression with Western blots and immunohistochemistry. Mechanically induced activation of ion channels was studied with pressure clamp, the Flexcell strain paradigm and optical imaging. Glial activation was studied in mice with conditionally ablated genes encoding putative mechanosensitive channels.

Electrophysiological and calcium imaging analyses showed that all classes of retinal glia express pressure-activated ion currents. TRPV4 mediated a significant fraction of the Muller response to pressure stimuli, swelling and matrix strain, which was reduced with pharmacological blockers and genetic deletion. Conversely, TRPV4 agonists stimulated reactive gliosis, microglial activation, and facilitated the release of gliotransmitters and cytokines in vitro and in vivo. Different glial populations appeared to be independently activated by mechanical stressors.

Our data provide experimental support for the hypothesis that elevated IOP acts on nonaxonal targets, and identify a novel mechanism that could drive functional loss of vision in glaucoma patients with therapeutically stabilized IOP.

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T03-006B

mTORC1 signaling regulates activity of Na^+/bicarbonate cotransporter 1, NBCe1, in mouse cortical astrocytes through phosphorylation of Ser^{245}

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The electrogenic sodium-bicarbonate cotransporter 1, NBCe1, is highly expressed in astrocytes and is involved in the modulation of intracellular pH, extracellular pH, and synaptic pH. Extracellular acidic environment is associated with a variety of pathophysiological conditions, among them ischemia and tumors. In addition, in epithelial cells, acidosis inhibits mTORC1 signaling. We have previously shown that in astrocytes, following metabolic alkalosis, NBCe1 functional expression is significantly downregulated, a process controlled by mTOR. Moreover, we have shown that m-TOR mediated phosphorylation of $Ser^{255-257}$ is positively correlated with NBCe1 activity. The aim of the present study is to investigate whether long-term metabolic acidosis regulates astrocytic NBCe1 and elucidate the underlying molecular mechanism.

Mouse primary cortical astrocytes were exposed to extracellular pH 7.4 or 6.8 for 3 h and regulation of NBCe1 was determined by immunoblotting, biotinylation of surface proteins, intracellular H^+ recordings, using pH-sensitive fluorescent dye (BCECF), and phosphoproteomic analysis.

During acidosis, NBCe1-mediated intracellular pH recovery from acidification was significantly increased, without differences in NBCe1 protein abundance or surface expression. Upon mTOR inhibition with rapamycin, NBCe1 activity was suppressed at both control and acidotic astrocytes. Phosphoproteomic analysis showed that NBCe1 is constitutively phosphorylated at Ser^{245}. Furthermore, mutational analysis uncovered that mTOR dependent phosphorylation of Ser^{245} upregulates NBCe1 activity.

Our findings demonstrate phosphorylation at Ser^{245} as a novel regulator of NBCe1 transport activity. Together with our previous work, the results implicate that mTOR-mediated multisite phosphorylation of NBCe1 maybe potent to fine-tune NBCe1 activity.

T03-007B

Microglial identity and inflammatory responses are controlled by the combined effects of neurons and astrocytes

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Microglia, brain resident macrophages, require instruction from the CNS microenvironment to maintain their identity, morphology, and to regulate inflammatory responses, although what mediates this is unclear. We hypothesised that neurons and/or astrocytes provide a surrogate CNS environment that promotes expression of

mature microglial signature genes, ordinarily lost when microglia are maintained ex vivo, as well as provide physiologically relevant immunomodulatory cues. We used a recently described approach of mixed-species RNA-seq: a tool for elucidating non-cell-autonomous control of gene transcription. Following the co-culture of purified neurons, astrocytes, and microglia from different species (mouse, human and rat, respectively), individual cell types' transcriptomes can be profiled simultaneously through the species-specific sorting of bulk RNA-seq reads using our Python tool *Sargasso*, avoiding gene expression artefacts and imperfections associated with physical sorting. Using this platform, we find that neurons and astrocytes combine to promote microglial signature gene expression ordinarily lost upon isolation ex vivo and lost in disease-the so-called microglial neurodegenerative phenotype. Neurons and astrocytes also combine to repress an interferon-related gene cluster that is associated with age and injury in vivo and is acquired when microglia are maintained in isolation, and also reduce microglial phagocytic capacity, consistent with their influence on the microglial transcriptome. These effects were recapitulated in a single species (all mouse) co-culture. We also performed single-cell RNAseq analysis using FAC-sorted rat microglia, which shows that neuron and astrocyte co-culture moves microglia into a distinct state in co-culture compared to microglia in mono-culture. We also discovered that secreted factors including TGF-b2 are required for astrocyte and neuron-dependent induction of microglial signature gene expression.In addition, neuron and astrocyte co-culture provides powerful modulatory cues that influence the dose-response of microglia to TLR4 activation by LPS, without affecting basal expression of LPS-response genes, nor their maximal response. Moreover, the transcriptomes of neurons and astrocytes are also modified by LPS-activated microglia in co-culture, consistent with in vivo data. These findings explain why microglia isolated ex vivo undergo de-differentiation and inflammatory deregulation, and point to how disease and age-associated changes may be counteracted.

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T03-008B

A role for astrocytes in the visual cortex critical period

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Information processing in the brain is generally considered as a neuronal performance. Yet, astrocytes play a crucial role in controlling synapse formation, maturation, activity and elimination[1], and thus shape synaptic circuits during development[2]. Remarkably, astrocytes regulate critical developmental periods, defined as periods of early postnatal development during which neuronal networks are highly plastic and sensitive to experience[3,4]. However how astrocytes control critical period development remains unknown.

Here using a multidisciplinary approach combining molecular biology, intrinsic optical imaging and electrophysiology, we show that astrocytes maturation contribute to the closure of the critical period in the mouse visual cortex. We uncover the underlying mechanism, which involves an unconventional astroglial connexin signaling pathway controlling the maturation of the extracellular matrix and interneurons.

Our results thus provide a novel unconventional astroglial pathway controlling the termination of the critical period, which is associated with settling of neuronal circuits. These data indicate that astrocytes play an essential role in the experience-dependent plasticity of brain developing circuits, and point to a novel cellular target to treat neurological disorders involving defects in the closure of critical periods.

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T03-009B

Morphology determines the calcium dynamics in astrocytes

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Astrocytes are involved in information processing and memory formation in the brain. An astrocyte participates in neuronal activity by receiving neurotransmitters, e.g., glutamate, from an adjacent presynapse. This leads to the propagation of intra- and intercellular calcium waves. However, it is not clear what effect the astrocyte's morphology has on these calcium dynamics. In this study, we present a finite-element-method-based model of single astrocytes with realistic 2D geometries. The computational model simulates the propagation of intracellular calcium waves after the stimulation by local glutamate. Following Oschmann et al. (2017), we include two spatially separated pathways of calcium release triggered by metabotropic glutamate receptors in the soma and glutamate transporters in the processes. We simulate several realistic astrocyte geometries with the same glutamate stimulus.

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T03-010B

Angiotensin II AT_1-cannabinoid CB_1 receptor heteromer implication in Parkinson's disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide, affecting about 1% of the population above 60 years. Moreover, due to aging population, PD prevalence is currently increasing. Current treatments aim at managing motor and nonmotor symptoms, but being unable to reduce the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*. It has been postulated that neuroinflammation might play a crucial role in the pathogenesis of PD. In fact, microglia transits from a resting state to an activated state when the nervous system undergoes pathological alterations such as dopaminergic neuronal death in PD. On the other hand, it is well-known that cannabinoid CB_1 receptor (CB_1R), the G-protein coupled receptor (GPCR) most expressed in the central nervous system, show a neuroprotection profile, thus being a target to reduce the chances to suffer from a side effect of anti-PD medication, namely dyskinesias. In addition, inhibition of angiotensin II type 1 receptor (AT_1R) protects against alpha-synuclein-induced neuroinflammation. Over the past decade, GPCR heteromers have been described as potential novel therapeutic targets. The aim of this work consisted of studying the heteromer formation between CB_1R and AT_1R in a heterologous system and to investigate its expression and function in both resting and activated microglia. Our results analysed by immunocytochemistry and resonance energy transfer assays show that CB_1R and AT_1 receptors colocalize in the plasma membrane and interact forming heteromers in transfected HEK-293T cells. In addition, as both receptors (AT_1R and CB_1R) may couple to Gi proteins, whose engagement leads to inhibit adenylate cyclase and decrease intracellular cAMP levels, we have detected, in both HEK-293T cells and activated microglia, a cross-antagonism phenomenon where the antagonists of AT_1R block CB_1R induced signalling and *vice versa*. In assays of ERK1/2 phosphorylation similar results were obtained. However, this phenomenon was not found in calcium released experiments of calcium mobilization (the AT_1R may also couple to Gq), as AT_1R activation was potentiated when forming complexes with CB_1R. Interestingly, activated microglia showed an increase in AT_1R-CB_1R heteromers expression. Finally, it has been found an overexpression of the AT_1R-CB_1R heteromer in samples form 6-hydroxy-dopamine-hemilesioned rats treated with chronic L-DOPA, and even more in rats showing dyskinetic problems. Taken together, we can conclude that AT_1R-CB_1R heteromer could be proposed as a new therapeutic target for Parkinson disease.

T03-011C

Kidins220/ARMS mediates astrocyte developmental switch in BDNF sensitivity, calcium signaling and neuron-astrocyte communication

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Astroglial cells are key to maintaining nervous system homeostasis. They are capable of perceiving a wide variety of extracellular cues and transducing them via the activation of specific intracellular signaling pathways into responses that may be protective or disruptive toward neighboring neurons. Moreover, glial cells are key regulators of neuronal circuit formation and activity through their ability to modulate synaptic transmission. Neurotrophins are a family of growth factors known for their pleiotropic effects on neuronal survival, maturation and plasticity. Additionally, they also control several aspects of astrocyte physiology. Kidins220/ARMS (Kinase-D interacting substrate of 220 kDa/Ankyrin repeat-rich membrane spanning) is one of the key effectors of neurotrophin pathways in neurons, where it is required for differentiation, survival and plasticity. However, its role in glial cells remains largely unknown.

In this work, we first compared the signaling competence of embryonic and postnatal primary cortical astrocytes exposed to brain-derived neurotrophic factor (BDNF) and found a shift from a kinase-based response in embryonic cells to a predominantly $[Ca^{2+}]_i$-based response in postnatal cultures. We found that Kidins220 ablation is accompanied by a decreased expression of the full-length and truncated BDNF receptor TrkB. We also describe the role of Kidins220 in BDNF-induced signaling in astrocytes, showing that it contributes to both kinase and Ca^{2+}-activated pathways. Kidins220 ablation induces defects in Ca^{2+} signaling that are linked to altered store-operated Ca^{2+} entry and strong overexpression of the transient receptor potential channel TRPV4. Moreover, embryonic Kidins220$^{-/-}$ astrocytes were more sensitive to genotoxic stress. We also show that Kidins220 expression in astrocytes is required for the establishment of proper connectivity of co-cultured wild type neurons.

Altogether, our data add to the understanding of the complex role played by astrocytes within the central nervous system by revealing a previously unidentified role for astrocytic Kidins220 in the control of glial responsiveness to BDNF, Ca^{2+} dynamics, survival/death pathways and astrocyte-neuron communication.

T03-012C

Vitamin K enhances functional modification of the pro-myelinating factor Gas6 in mouse brain glial cells

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Background

Multiple sclerosis (MS) is an autoimmune neuroinflammatory disease which targets the myelin sheath of CNS neurons for damage. The vitamin K-dependent protein (VKDP) Gas6 has been shown to stimulate CNS myelination. Gas6 functionality depends upon its post-translational modification in a vitamin K (VK)-driven process, involving several enzymes of the VK cycle. However, whilst this system is well characterised in the liver, relatively little is known about the role of VK in the brain. The aim of this study is to determine the presence and functional role of the VK-dependent activation of Gas6 in the mouse brain, including in distinct brain glial cells as well as throughout development.

Methods

Mouse whole brain and liver tissue was obtained at different developmental ages: embryo, postnatal ages P0, P7, P14 and adult. All procedures were carried out with approval from UK Home Office and local ethics committee. In vitro primary cultures were set up of neonatal mouse brain glial cells, including mixed glial cultures and pure microglia and astrocyte cultures. Expression of genes and proteins of the VK cycle in brain tissues and cells were measured by RT-qPCR and western blot. To determine VK effect on Gas6 protein released by glial cultures, cells were first incubated with the VK antagonist warfarin for 24hrs, after which VK was added to cells and incubated further for 48hrs. The medium was then collected for measurement of γ-carboxylated Gas6 (specific VK-dependent modification) by ELISA and western blot.

Results

The genes for the VK cycle enzymes *Nqo1, Ggcx, Vkorc1* and *Ubiad1* were increased in the liver throughout postnatal development, whereas the brain expression levels were lower and less varying. A key difference was the higher brain expression of *Vkorc1l1*, a paralogue of *Vkorc1* which is more prominent in the liver. Also, western blot analysis revealed expression of GGCX and VKORC1 proteins in mouse glia and brain extracts respectively.

In all glial cultures, warfarin virtually eliminated the presence of γ-carboxylated Gas6 in the cell medium as measured by ELISA. However, subsequent addition of VK effectively antagonised the warfarin and induced a robust expression of γ-carboxylated Gas6. The effect of VK was greater in microglia than in astrocytes, as was expression of total Gas6 in the medium.

ConclusionThese results show that there is a functional VK cycle in the mouse brain, as observed through expression of the key VK cycle components as well as the ability of VK to functionally modify Gas6 in brain glial cells. Therefore, these data have further elucidated the mechanisms behind the role of Gas6 in the brain in relation to neuroinflammatory disorders such MS.

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T03-013C

GPR17 expression is regulated by Wnt pathway through ID2 up-regulation during oligodendrocyte precursor cells differentiation

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Oligodendrocytes (OLs) differentiation is regulated by a complex interplay of intrinsic, epigenetic and extrinsic factors, including Wnt and the G protein-coupled receptor GPR17 [1]. GPR17 responds to both extracellular nucleotides (UDP, UDP-glucose) and cysteinyl-leukotrienes [2], endogenous signaling molecules involved in inflammatory response and in the repair of brain lesions. GPR17 is highly expressed in oligodendrocyte precursor cells (OPCs) during the transition to immature OLs, but it is down-regulated in mature cells. In cultured OPCs, early GPR17 silencing has been shown to profoundly affect their ability to generate mature OLs [3,4]. Understanding the signalling pathways modulating GPR17 expression is of utmost importance to fully understand the myelination process in normal and pathological conditions. Given this, very little is known regarding its transcriptional modulation. Here we analysed the previously unexplored interplay between the activation of the canonical Wnt pathway and GPR17 expression.

Firstly, we observed that *Gpr17* and Lymphoid enhancer-binding factor-1 (*Lef1*), a downstream mediator of the Wnt/β-catenin signalling pathway, were differentially expressed in PDGFRα+ OPCs and O4+ OLs during mouse brain development. Indeed, whereas *Gpr17* mRNA level peaked in PDGFRα+ and O4+ cells respectively at P7 and P14 and then decreased, *Lef1* was maintained in both populations at low and constant level till P10 and then progressively increased along with development. *In vitro* forced activation of Wnt/β-catenin pathway during OPC differentiation significantly up-regulated *Lef1* mRNA, which in turn induced GPR17 downregulation and OPC differentiation arrest. Luciferase experiments further confirmed that activation of Wnt/β-catenin pathway reduced *Gpr17* promoter activity. This inhibitory effect was partially rescued when the two consensus binding sites for TCF/LEF on *Gpr17* promoter (corresponding to potential Wnt response elements, WREs) were mutated or after silencing of Inhibitor Of DNA Binding 2 (*Id2)*, a negative regulator of OLs differentiation which is up-regulated by Wnt.

In conclusion we demonstrated that a lower Wnt activation tone is necessary when *Gpr17* expression is sustained whereas higher Wnt signalling results in a decrease in *Gpr17* expression during development. Moreover, Wnt pathway reduces *Gpr17* expression by a dual mechanism: directly through the interaction with WREs located on its promoter and by inducing *Id2* up-regulation which in turn downregulates the receptor expression. Disturbances in this delicate balance is associated with alterations in *Gpr17* expression and with a blockade of OPC maturation and could represent an unexplored pathological mechanism contributing to demyelination observed in several diseases.

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T03-014C

Molecular-physiological properties of ER calcium dynamics and calcium oscillations in cultured astrocytes

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Calcium homeostasis is of fundamental importance for many physio- and pathophysiological processes. To investigate homeostatic calcium fluxes, cultured astrocytes became an often-used cellular model. Here, we applied dual color calcium imaging (ER/cytosol) with reference to whole transcriptome data of cultured astrocytes to better describe molecular-physiological features of ER calcium dynamics.

Our data show rather complex ER calcium responses in cultured astrocytes despite a small calcium toolkit. The best candidate for depolarization-dependent calcium influx was the P/Q-type voltage-gated calcium channel Cacna1a, which is abundantly expressed in these cells. Depolarization of astrocytes with potassium led to rather different and complex depolarization-dependent calcium influx transients and these signals could be blocked by the P/Q-type antagonist agatoxin. ER-cytosol calcium oscillations could persist in absence of extracellular calcium and in presence of agatoxin, indicating that voltage-dependent calcium influx is not needed to induce or maintain calcium oscillations. Spontaneous calcium oscillations in the ER lumen and the cytosol are shaped by a non-linear, circular, or spiral relationship between both signals. The adenosine receptor Adora1a was found to be the most abundantly expressed metabotropic receptor. Indeed, low amounts of adenosine (1 – 10 μM) were sufficient to induce fast ER calcium release and ER calcium oscillations. The phospholipase b (*Plcb3*) is the best, or even only, candidate for mediating IP_3 production via metabotropic receptors. The mediators of stimulated ER calcium release are two IP_3 receptors (Itpr1/2), while ryanodine receptors (Ryr1-3) were virtually absent. The only candidate for the refilling of the ER calcium store is the SERCA *Atp2a2*. Blockade of the SERCA with Thapsigargin led to immediate emptying of the ER calcium store, pointing out the high speed of ER calcium leak. Homeodynamic calcium influx is most likely Stim1/2 – Orai1/2/3-dependent and Trp ion channels were barely expressed. We suggest a model in which the minimal requirement of spontaneous calcium oscillations are a pronounced ER calcium leak and calcium-dependent activity of the SERCA. In this concept, calcium influx or efflux over the plasma membrane are only modulators, but no mediators of oscillatory ER calcium activity.

T03-015C

Optogenetic activation of RAF pathway promotes astrocytogenesis in a timepoint-dependent manner

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Optogenetic takes advantage of light-sensitive proteins to dissect the function of molecular pathways. Utilizing the light-sensitive protein pair CRY2/CIB1, we have constructed the OptoRAF1 tool, in which RAF1 is recruited to the plasma membrane and activated upon blue light illumination, and apply it to interrogate the temporal role of the RAS/RAF/MEK/ERK pathway in gliogenesis. We found that light-induced OptoRAF1 activation in neural progenitor promotes cell proliferation and increased expression of glial markers and glia-enriched genes. However, Insufficient or delayed OptoRAF1 activation failed to promote astrocytogenesis. In addition, activation of OptoRAF1 did not have a significant effect on neurogenesis, but it promotes neuronal neurite growth. Apart from OptoRAF1, we have also devised several other optogenetic tools to manipulate the activation of Akt pathway, Wnt/beta-catenin pathway, PTEN pathway and so on, allowing us to gain deeper insight into how these pathways involved in neurodevelopment.

T03-016D

Deciphering spatio-temporal Ca^{2+} activity in astrocytes changes using multi-threshold event detection (MTED)

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Recent achievements in indicator optimization and imaging techniques promote the exploration of Ca^{2+} activity patterns. Astrocytes are important regulators of the brain network and well known for their highly complex morphology and spontaneous Ca^{2+} activity (Fig. 1). However, the astrocyte community is lacking standardized methods to analyze and interpret Ca^{2+} activity recordings, hindering global comparisons. Here, we present MTED (Multi-Threshold Event Detection) [1], a biophysically based concept to analyze astrocytic Ca^{2+} activity, which includes multiple thresholds and allows a differentiated and in-depth characterization of Ca^{2+} signal complexity. We analyzed various *ex vivo* and *in vivo* imaging datasets and verify the validity of our algorithm across Ca^{2+} indicators, imaging setups, and model systems from primary cell culture to awake, head-fixed mice. We found the Ca^{2+} activity patterns to be temperature-dependent across models and defined the subset of Ca^{2+} events shaped by neuronal impact. Applying our concept enables standardized analysis and advances astrocyte research for decrypting brain function.

Acknowledgement

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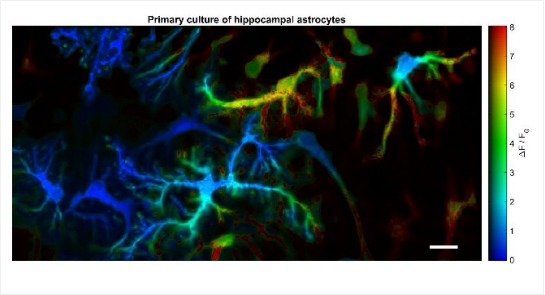

Fig. 1: Primary culture of hippocampal astrocytes expressing the Ca2+ indicator GCaMP6s.
Shown is the maximum projection of endogenous Ca^{2+} activity of a 10 min recording, visualized as $\Delta F/F_0$.

T03-017D

Deciphering the role of mitochondria-ER contactsin reactive astrocytes

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Astrocytes play an essential role in managing neuronal and synaptic support, blood-brain-barrier maintenance and neurovascular coupling. Most of these diverse functions in astrocytes are facilitated by a highly regulated Ca^{2+} activity, which presents itself with local transients of varying sizes. Importantly, Ca^{2+} activity in perivascular and perisynaptic astrocytic processes contributes to vascular tone and modulates synaptic transmission in mice *in vivo*. However, how these Ca^{2+} domains are generated, maintained and eventually reorganized with respect to physiological or pathological conditions, remains poorly understood.
Recently, we have identified the relevance of two essential intracellular organelles – namely mitochondrial and ER networks, that shape Ca^{2+} activity in perivascular astrocytic end-feet *in vivo*. Ultrastructural imaging revealed a natural enrichment of mitochondria-ER contacts (MERCs) in astrocytic end-feet, which became even more pronounced in injury-induced reactive astrocytes. Moreover, our study showed that conditional astrocytic deletion of the pro-fusion and pro- tethering protein Mitofusin 2 (Mfn2) was sufficient to alter the extent of MERCs in mice *in vivo*, impair mitochondrial Ca^{2+} uptake and lead to aberrant cytosolic Ca^{2+} transients within astrocytic end-feet. Importantly, the aberrant cytosolic Ca^{2+} transients, impaired mitochondrial Ca^{2+} uptake and flawed vascular remodeling displayed in $Mfn2^{cKO}$ mice could be rescued by manipulations enforcing the extent of astrocytic MERCs. These observations prompted us to investigate the dynamics of astrocytic MERCs *in situ* and *in vivo*. Given that MERCs are important sites for Ca^{2+} transfer, we will introduce genetic manipulations targeted at the mitochondrial Ca^{2+} uptake machinery to further dissect the role of mitochondrial Ca^{2+} signaling in mediating astrocyte reactivity. Utilizing a combination of mouse genetics, stereotactic viral injections, and imaging techniques, we aim to elucidate the mechanisms which control the establishment and dynamic reorganization of astrocytic MERCs – in physiological conditions, as well as in response to brain injury.

T03-018D

Astrocyte mitochondrial structure and dynamics in physiological and pathological conditions

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The structural and Ca2+ dynamics of mitochondria in astrocytes during physiological and pathological conditions are not well understood. To study mitochondria dynamics in astrocytes, we used the GLAST-CreER;mito-EGFP double transgenic mouse line, in which EGFP is targeted to the mitochondrial matrix of astrocytes. These mice were exposed to a small amount of tamoxifen to label mitochondria in a sparse population of astrocytes. In the adult GLAST-CreER;mito-EGFP mice we implanted chronic glass window over the somatosensory cortex and performed 3D time-lapse 2-photon microscopy to image mitochondria in individual astrocytes at various time-scales, ranging from hours to several weeks. We observed that mitochondria in astrocyte are located in the soma, main processes and in the fine branches of these processes. To automatically 3D segment mitochondria and to characterize their structural and Ca2+ dynamics, we developed a machine-learning based algorithm in Ilastik and MATLAB, called mito-CaSCaDe. Astrocytic mitochondria showed a reduced motility, fusion and fission dynamics, and formed dense mitochondria networks, which are stable over several days. Moreover, Focused Ion Beam-Scanning Electron Microscopy (FIB-SEM) data analyses, confirmed the presence of a highly connected mitochondria network in astrocytes processes, which encompasses a considerable volume of the astrocyte cytoplasm. To study mitochondrial Ca2+ signals, we used the AAV based viral approach to express a mitochondrial targeted genetically encoded calcium indicator GCaMP6s in cortical astrocytes. In acute brain slices derived from adult mice, we observed that mitochondria show spontaneous fluctuations in matrix Ca2+, and the bath application of neuromodulators such as ATP and norepinephrine induced Ca2+ transients in mitochondria. Our ongoing studies will further yield insights into the role of astrocyte mitochondria in modulating astrocyte energetics and physiology.

T04 | Cytoskeleton

T04-001D

Injury induced Drebrin controls astrogliosis and scar formation by regulating tubular endosomes and adhesion responses

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The Central Nervous System (CNS) of higher mammals is particularly vulnerable to traumatic injuries and diseases but lacks a significant capacity to regenerate lost neurons and, consequently, to regain the functionality of damaged brain areas. Therefore, an immediate and persistent containment of any locally occurring pathological incident is required to avoid the propagation of inflammation and neurodegeneration into the uninjured brain parenchyma.

In the present study, we provide a novel perspective and mechanistic insight regarding the function of drebrin as injury-specific actin regulator for polarized membrane trafficking and adhesion responses. We show that mechanical injuries *in vitro* and *in vivo* induce in astrocytes an *ad hoc* upregulation of Drebrin, which is required for the coordinated formation of glial scars. On the molecular level, Drebrin deficiency affects trafficking of Rab8-positive tubular endosomes and leads to their disruption. Dispersed Rab8 impairs retrograde membrane trafficking, evoking the mislocalisation and failed turnover of Integrin beta1. We propose a model for Drebrin in orchestrating actin dynamics on tubular endosomes to enable polarized membrane trafficking and controlled turnover of crucial receptors, which reactive astrocytes require to form glial scars and protect the sensitive CNS from traumatic brain injuries *.

Acknowledgement

We thank Magdalena Götz and Sofia Grade (LMU Munich) for the provision of slices from the BAC Aldh1l1:GFP reporter mice, the adavanced medical bioimaging core facility (AMBIO) for usage of the facility. Funding was provided by the DFG (SFB 958 and 'Sachbeihilfe'- Project 285933818, NeuroCure EXC257); Sonnenfeld-Stiftung (J.S.).

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T04-002D

A trend of evolution: expansion of GFAP free brain areas, and its appearance in Primates

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Surveying the former results, a general tendency emerges: GFAP-poor or almost free areas appear and expand (Kálmán, 2002). These areas usually represent new acquisitions during evolution. Note that lack of GFAP does not mean lack of astroglia, since some subtypes express GFAP only facultatively or not at all. The bulky, thick-walled telecephalon of batoids contains astroglia only scarcely as compared to the shark telencephala which have relatively large ventricles and thin walls. Among aqctinopterygii cyprinids developed a large and sophistically layered gustatory center (vagal lobe). The layers of sensory and motoric neurons are free of GFAP. These layers are not found in less advanced gustatory centers, e.g. in sterlet. Avian homologues of some GFAP-rich turtle or *Caiman* brain areas were almost free of GFAP, e.g. the molecular layer of the cerebellum, the superficial layers of the tectum (Fig. 1), and most of dorsal ventricular ridge. Among Squamata the GFAP immunopositivity can extend over the whole telencephalon (geckos, monitor lizards) or only a part of it (lacertids, snakes), or only represented in confined areas (chameleons, agamas). The entopallium is intensely GFAP-immunopositive in the older groups of birds (Galloanserinae, Columbiformes) but negative in songbirds. In mammals the paleocortex is GFAP-immunopositive in full thickness, whereas in the neocortex the middle layers are very poorly immunopositive (Fig. 2). Most of thalamic nuclei are negative, except for the reticular nucleus. These distributions were also found in Primates (*Saimiri* and *Cebella* species). A regression of intermediate filaments also takes place during ontogenesis, since the embryonic brain is still completely colonized by vimentin-immunopositive radial glia in both mammals and birds. Lesions provoke GFAP expression in the GFAP-negative areas in birds and mammals but not in other Amniotes (Lőrincz and Kálmán, in press). It suggests that the GFAP production is only repressed in the former groups but missing in the latter group in these areas. We suppose that the lack of GFAP underlies the plasticity of 'modern' brain areas, and seems to be an apomorphic feature of an area.

Acknowledgement

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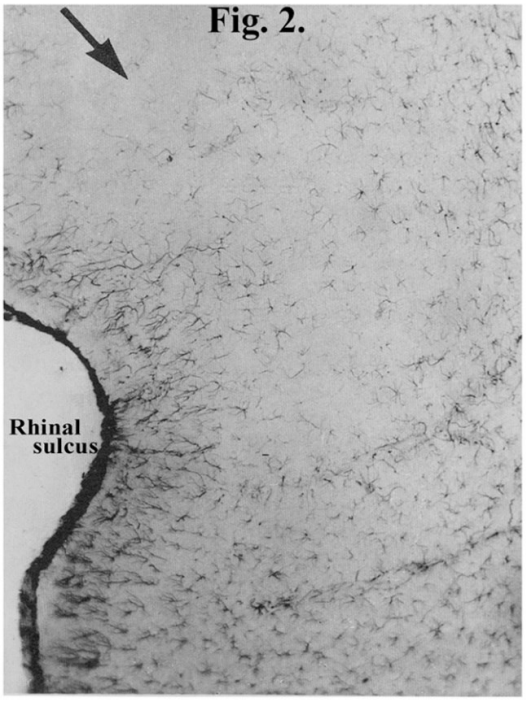

Fig. 2. GFAP distribution in rat cortex.
In the middle zone of neocortex (arrow) the GFAP+ astrocytes are very scarce, in contrast to the paleocortex (below the rhinal sulcus).

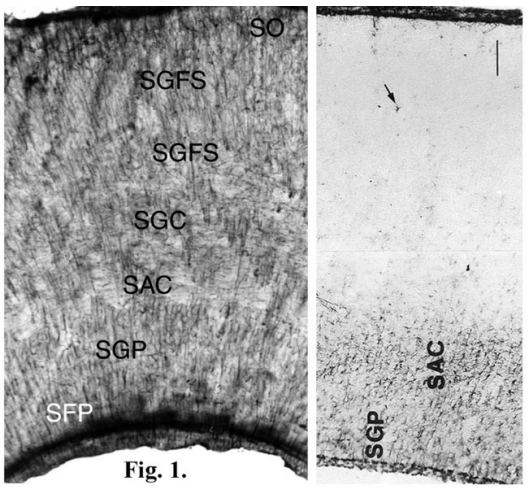

Fig. 1. GFAP distribution in turtle and chicken tectum
Note the diference (the turtle is to left). SFP, etc. - the tectal layers. Arrow points to a single GFAP+ astrocyte.

T04-003D

GFAP regulates mitochondrial distribution and function in astrocytes

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GFAP is a type III intermediate filament highly expressed in mature astrocytes. While GFAP is used as an astrocyte marker for decades, the function of GFAP besides the role of providing tensile strength is largely unknown. A fatal disease called Alexander disease (AxD), caused by GFAP mutation, provides us an opportunity to study the role of GFAP at the cellular level. We have generated induced pluripotent stem cells (iPSCs) from AxD patients with C88 and W416 mutations and their isogenic controls via genetic correction by CRISPR. Astrocytes derived from the AxD iPSCs display GFAP aggregation, recapitulating the characteristic AxD pathology. Interestingly, in AxD astrocytes organelles such as ER and mitochondria are redistributed irregularly throughout the cell body as opposed to radial arrangement from the nucleus to cell processes in isogenic astrocytes. In particular, almost all mitochondria in disease astrocytes are short or fragmented in contrast to coexistence of both long and short mitochondria in isogenic controls. Functionally, mitochondria in AxD astrocytes exhibit unstable transient membrane potentials and produce more ROS than in control astrocytes, and these phenotypes are reversed once GFAP mutation is corrected, suggesting the cause-effect relation between mutant GFAP and dysregulated mitochondria function. By expressing WT-GFAP or C88 mutant-GFAP in RBL-2H3 cells or fibroblasts, which do not have endogenous GFAP, or human astrocytes with GFAP knockout, we observed that WT-GFAP is able to form a filamentous structure while mutant GFAP is prone to accumulate at a high expression level. Photobleaching (FRAP) and optogenetic analyses reveal that the mutant GFAP is dynamic (fragile) as compared to the stable filamentous WT-GFAP. Ongoing studies aim to identify how GFAP regulates mitochondria organization, distribution, and function.

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T04-004D

WAVE1 and N-WASP in axon ensheathment and myelin formation

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Oligodendrocytes (OLs) ensheath axons in the central nervous system with myelin. Myelin speeds up action potentials and provides metabolic support to axons. Myelination is a highly specialized cell-cell interaction and the mechanism of OLs ensheathing axons involves dynamic changes to the actin cytoskeleton. However, the molecular mechanisms of OLs extending processes to make the initial axonal ensheathment remain unclear. Two actin scaffold proteins, Wiskott–Aldrich syndrome protein family member 1 (WAVE1) and Neural-Wiskott Aldrich Syndrome protein (N-WASP), bind to and activate the Arp2/3 complex, the master regulator initiating actin filament formation. Germline knockout (KO) of WAVE1 in mice alters neuronal morphology and also results in hypomyelination, enlargement of ventricles, reduced OL number and decreased size of white matter tracts such as

the corpus callosum. Deletion of N-WASP in pre-myelinating OLs by crossing N-WASP (flox) with Cnp-Cre mice, results in abnormal myelin formation. We deleted WAVE1 in all stages of the OL lineage by crossing WAVE1(flox) with Sox10-Cre mice. Immunolabeling for Myelin basic protein (Mbp) showed no sign of hypomyelination, nor was there any reduction in the number or density of Olig2-positive OL lineage cells or CC1/Olig2 double-positive OLs. Together these results indicate that WAVE1 ablation in the OL lineage does not affect OL generation or myelination. We conclude that the myelin defects observed in germline WAVE1 KOs are a secondary consequence of neuronal loss. Since both WAVE1 and N-WASP act upstream of Arp2/3, we hypothesize that N-WASP might compensate for the absence of WAVE1 in OLs. To test this hypothesis we are generating WAVE1/N-WASP double-KOs and predict that OLs will be unable to form the first ensheathing wrap around axons in these mice.

T04-005D

PAK1 regulates oligodendrocyte membrane formation and myelination

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In the central nervous system, myelin formation by oligodendrocytes (OLs) depends on actin dynamics. The ensheathment step of the myelination process is driven by actin polymerization, while the wrapping phase requires a switch to actin depolymerization. The molecular mechanisms regulating this switch are not fully understood. The P21 activated kinase 1 (PAK1), a downstream effector of the Rho GTPases Rac and Cdc42, can regulate actin polymerization balance through its kinase activity. We showed that the expression of PAK1 increases throughout the oligodendroglial differentiation and maturation while its kinase activity decreases, resulting in actin disassembly through its downstream effectors. Using *Pak1* conditional knockout mice in OLs (*Pak1* cKO) and *in vitro* knockdown experiments, we showed that PAK1 controls membrane formation and myelination. Indeed, morphological analysis, based on MBP expression and F-actin staining, revealed that the formation of OLs with established membranes and disassembled F-actin increases under *Pak1* knockdown condition. Likewise, the expression of cofilin and gelsolin, which regulate actin disassembly, significantly changes in OLs with *Pak1* knockdown. Furthermore, pharmacological inhibition of PAK1 by a highly specific inhibitor (NVS-PAK1-1), triggers OL membrane extension and myelination *in vitro* and *ex vivo*, respectively**.** Overall, our data highlight PAK1 as a key regulator of OL membrane growth and myelination through actin cytoskeleton remodelling.

T05 | Degenerative disease, toxicity and neuroprotection

T05-002A

Synapse Loss in Progressive Supranuclear Palsy and Corticobasal Degeneration and the Role of Astrocytic Tau

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Introduction

The primary 4-repeat tauopathies progressive supranuclear palsy (PSP) or corticobasal degeneration (CBD) share characteristic, neuropathological traits comprising neurofibrillary tangles (NFT), neuropilic threads (NT) and oligodendroglial coiled bodies (CB) of accumulated hyperphosphorylated tau (pTau) in cerebral areas among other locations. Astrocytes, however, contain pathognomonic formations of pTau aggregates, namely tufted astrocytes (TA) in PSP or astrocytic plaques (AP) in CBD. The role of neuronal pTau has been shown to be associated with synaptotoxicity, though the role of glial pTau inclusions and their implications for neurotransmission in human tauopathy brains remains incompletely understood.

We hypothesized, that the extent of neuropathological traits relates to reduced numbers of pre- and/or postsynapses in the cerebral cortex of PSP and CBD individuals.

Methods

To capture morphometric synapse alterations, we performed immunofluorescent synapse labeling (excitatory vGLUT1+/HOMER1+; inhibitory vGAT+/GEPHYRIN+) and automated puncta quantification in the frontal Cortex (fCtx) and striatal regions (Caudate nucleus, Globus pallidum; Str) from PSP (n=3), CBD (n=3) and Ctrl (n=3) autopsy cases. To assess potential correlations of synaptic density with the extent of cellular pTau pathology, single traits were quantified as total numbers per microscopic visual field (TA/AP, NFT, CB; 250x) or graded semi-quantitatively (NT). Astrocytic domains of TAs/APs and controls were analyzed using “Sholl-like area representations”, within which synaptic puncta were quantified.

Results

Significant synaptic loss of both excitatory and inhibitory bipartite synapses in the frontal cortex of PSP cases without significant trait associations were observed (Figure 1a,c). In CBD, a lower synapse density was only observed at astrocytic plaques. In the astrocytic domain-centered analysis, single APs displayed an abnormal synapse distribution at principally reduced density levels, while TAs exhibited only minor changes within the most proximal part of their synaptic islands, when compared to non-affected astrocytes close by or astrocytes of control patients (Figure 2).

Discussion

In summary, these findings support the notion that astrocytes support synapse function, which might be disturbed in close vicinity of astrocytes with CBD-typical tau pathology. In CBD this may contribute to cognitive dysfunction, however, in PSP there is no obvious trait association. Through juxtaposing neuropathological trait associations of synapse loss in both tauopathies, we emphasize astrocytic pTau as a potential cellular component in understanding the pathophysiology in CBD rather than in PSP.

Acknowledgement

We would like to thank the patients and their families for enabling our research through brain tissue donation. We would also like to thank the *Neurobiobank Munich* members for conceptual contributions. This work was funded by the *Munich Cluster of Systems Neurology* (*SyNergy*; project ID EXC 2145 / ID 390857198), LMU Munich, Germany. Personal funding included *German Academic Scholarship Foundation* (NB) and *Marie Sklodowska-Curie actions* ITN *SynDegen* (ID 721802; KP).

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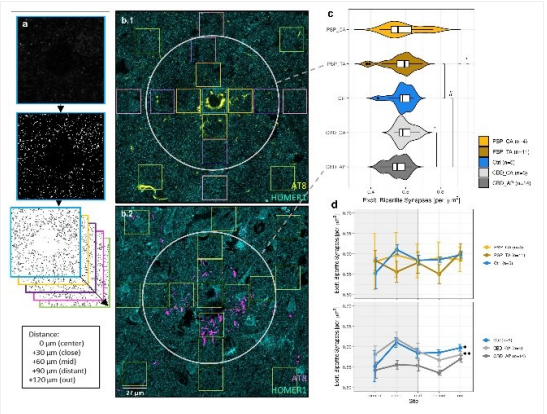

Figure 2. Derogated synaptic islands in astrocytes of APs.

a Overview of the astrocytic domains analysis. Raw images (squares in b) are preprocessed before automatic puncta detection. Five distances are measured (*center, close, mid, distant, out*).

b *Ex vivo* confocal images of TA (1) and AP (2). The order of bins on concentric circles is shown as color code. The white circle delineates the *a priori* size of an astrocytic domain.

c Violinplots of comparisons of astrocytic domain-attributed bins. Pair-wise Welch's t-tests.

d Lineplots of synapse densities across area representations stratified by disease and tau pathology.
P-values: #p<.1, *p<.05, **p<.01

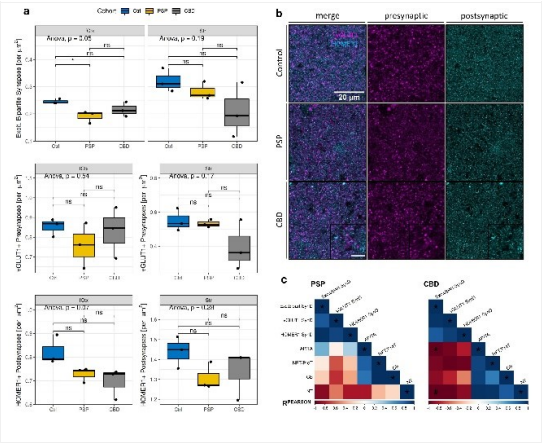

Figure 1. Synapse loss in PSP fCtx and CBD.
a Boxplots of the synaptic densities for colocalized puncta$^{vGLUT1+/HOMER1+}$, presynapses$^{vGLUT1+}$ and postsynapses$^{HOMER1+}$ in the fCtx (left) or the Str (right column). Group entities indicated as color code. Pair-wise Welch's t-tests and ANOVA.

b *Ex vivo* confocal images of exc. synapses in the fCtx of Ctrl (1st row), PSP (2nd row) and CBD subjects (3rd row) using vGLUT1 and HOMER1 as pre- or postsynaptic markers.
c Visualization of Pearson-correlation matrices between synapse densities (SynD) and traits (TA, NFT, CB, NT) in the fCtx.
P-values: ns = "not significant", #p<.1 and *p<.05

T05-003A

Can manipulation of neuroinflammation modulate oligodendrogenesis and white matter repair after TBI?

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Introduction

There is an unmet clinical need to develop greater mechanistic understanding of white matter injury after traumatic brain injury[1]. High-mobility group box 1 protein (HMGB1) is implicated as a regulator of brain inflammation following neurotrauma, and elevated levels of it in the CSF and serum of TBI patients is associated with worse outcomes. This study investigates the effect of HMGB1 on oligodendrocyte progenitor cells (OPCs) in an *in vitro* model of TBI.

Methods

We subjected rat cortical mixed neuro-glial cell cultures (7DIV) to standard scratch injury as described elsewhere[2,3]. Fresh cortical cell cultures were then maintained under standard control conditions, control conditioned media, or injury conditioned media from the scratched cultures, in the presence or absence of the HMGB1 receptor (TLR 2/4) antagonist BoxA. Immunofluorescence microscopy for $NG2^{+}$ (OPCs) was performed to ascertain cell counts. Levels of HMGB1 were measured in condition media and at 4 and 6 hrs post injury using ELISA kits (R&D systems & IBL International).

Results

Our data demonstrated a statistically significant increase in HMGB1 concentration within injury conditioned cell media (ICM) at 6 hours post injury verses control conditioned media (CCM) (17.1±1.4 vs 23.2±0.1ng/ml; $p = 0.02$, Fig. 1). Treatment of fresh cortical cells with ICM (Fig. 2a) resulted in a significant decrease in $NG2^+$ cells compared to control conditions (59cells/mm2 ± 5.8 SE vs.16cells/mm2 ± 1.9; $p < 0.0001$). Co-treatment with BoxA did not affect $NG2^+$ cell counts in the control or CCM conditions, but abolished ICM-induced $NG2^+$ cell loss (16cells/mm2 ± 1.8 SE vs. 85cells/mm2 ± 14.0; $p < 0.0001$). Astrocyte cell counts were not affected by the application of ICM (Fig 2b).

Conclusions

$NG2^+$ cell counts were significantly reduced upon application of ICM to cell cultures. Since $NG2^+$ counts were rescued upon inclusion of BoxA in culture, this effect is likely acting through interactions with TLR2/4 receptors. This implicates HMGB1, which we demonstrated is released into ICM in this TBI model, as a modulator or OPC survival and/or proliferation. This effect was not seen with astrocytes, suggesting that HMGB1 may be a specific modulator of OPCs, and hence white matter repair, after neurotrauma. Identification of the toxicity of *in vitro* scratch injury to OPCs, which is reversed upon blockade of HMGB1-signalling, suggests that HMGB1-mediated pathways may be a potential therapeutic target for improving TBI-associated morbidity.

Acknowledgement

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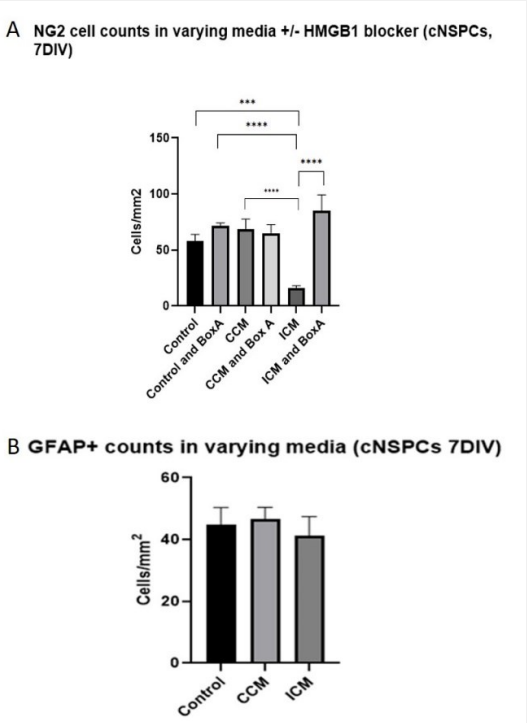

Figure 2
A: NG2 cells were significantly reduced in the presence of injury conditioned media (ICM) compared to control and control conditioned media (CCM). BoxA (100ng/ml) did not affect NG2 cell counts in control or CCM. However, BoxA rescued NG2 cell counts in the presence of ICM to levels similar to control cultures ($p < 0.0001$).

B: Astrocyte cell counts were not affected by the application of CCM or ICM compared to control conditions. Two-way ANOVA (Dunnett's multiple comparison test) was used. p values of <.05 were considered significant (****, $p<0.0001$). cNSPCs=cortical neural stem cell progenitors.

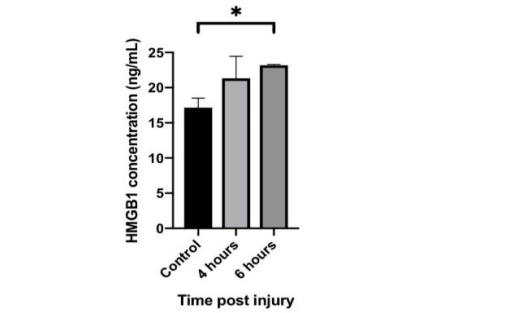

Figure 1
Figure 1. HMGB1 is released into culture media following in vitro scratch injury. ELISA revealed significant increases in extracellular HMGB1 concentration at 6 hours post-injury. Data represent mean ± standard error based on a sample that represents at least 10 wells per condition from three different experiments. One way and two-way ANOVA with Dunnett's multiple comparison test was used. p values of < 0.05 were considered significant (*, $p < 0.05$; **, $p < 0.01$). cNSPCs = rat cortical neural stem cell progenitors; DIV = days in vitro

T05-004A

Conservation and divergence of vulnerability and responses to stressors between human and mouse astrocytes

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Astrocytes play important roles in neurological disorders such as stroke, injury, and neurodegeneration. However, the similarities and differences between human and mouse astrocytes are largely unknown. As human-mouse differences are a major barrier in translational research, we investigated the conservation between human and mouse astrocytes. Based on analyses of acutely purified astrocytes, serum-free cultures of primary astrocytes, and xenografted chimeric mice, we found extensive conservation in astrocytic gene expression between human and mouse samples. However, the genes involved in defense response and metabolism showed species-specific differences. Human astrocytes exhibited greater susceptibility to oxidative stress than mouse astrocytes, due to differences in mitochondrial physiology and detoxification pathways. In addition, we found that mouse astrocytes but not human astrocytes, activate a molecular program for neural repair under hypoxia, whereas human astrocytes but not mouse astrocytes, activate the antigen presentation pathway under inflammatory conditions. These species-dependent properties of astrocytes may contribute to differences between mouse models and human neurological and psychiatric disorders.

Acknowledgement

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T05-005A

The use of arundic acid as novel therapy for multiple sclerosis

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In recent years, numerous studies have investigated the neuroprotective effects of Arundic Acid (AA), which is known to inhibit the astrocytic synthesis of the alarmin S100B in animal models of central nervous system (CNS)diseases such as cerebral ischemia, Alzheimer's and Parkinson's diseases (for review, 1). In the light of recent findings indicating a protective role exerted by Pentamidine, which is known to block S100B action, in

Multiple Sclerosis (MS) (2), we explored a possible role of AA aiming for novel effective therapeutic solutions for MS. The administration of AA in mice affected by experimental autoimmune encephalomyelitis (chronic progressive form, P-EAE) was able to block or delay the onset of the acute phase of the disease, as well as to decrease the intensity of symptoms and improve biomolecular and histopathological parameters.

Specifically, the treated P-EAE group of mice showed lower severity of cumulative disease score compared with vehicle-treated mice, and particularly in the early phase of disease onset that includes the first acute phase and the onset. The measurement of enzymatic activity of NOS and ROS in P-EAE mice showed that the treatment with AA has an antioxidant effect decreasingROS and NOS to control levels. Quantitative PCR assay performed in total mRNA samples extracted from different brain areas of mice showed a reduction of cytokines IL1b and of S100B in AA-treated compared to untreated P-EAE animals. The ability of AA to modulate S100B was particularly evident in posterior brain regions as demonstrated also using ELISA assay.

We also performed morphological studies to dissect the impact of AA on different areas of the brain during P-EAE, specifically addressing astrocytosis (GFAP staining), demyelination (MBP), microglia activity (CD68 and Iba1), presence of infiltrates (CD4). The treatment with AA was efficient in the control of astrocytosis and demyelination at different districts of CNS, while it downmodulated infiltrates and microglia activation particularly in the cerebellar areas and in spinal cord.

In the light of these effects, AA might show effective applications in the prevention and/or treatment of MS, especially when administered during the acute phase of the disease, concomitant with the blood-brain barrier damage that is known to accompany this phase, facilitating the passage to the nervous tissue of the drug.

Acknowledgement

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Disclosures: GDS, FM; SA, MEC, BS, FR declare to be inventors of a submitted patent (102020000015895) entitled “The use of arundic acid in the therapy of multiple sclerosis”. The other Authors declare no disclosures.

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T05-006A

Alexander disease modeling in zebrafish as an in vivo system suitable to perform drug screening

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Alexander disease (AxD) is a rare astrogliopathy caused by heterozygous mutations in the glial fibrillary acid protein (GFAP) gene, encoding the glial intermediate filament. Mutations in the GFAP gene makes the protein prone to form aggregates which, together with HSP27, □B-crystallin, ubiquitin and proteasome, contribute to form Rosenthal fibers causing a toxic effect in astrocytes. Unfortunately, no pharmacological treatment is available yet, except for reducing-symptoms therapies, and patients undergo a progressive and fatal worsening of the disease. In vitro models of AxD, consisting in transient expression of mutant GFAP protein in cell cultures, suffer from limitations compared to animal organisms, with consequently arising difficulties in studying complex biological processes, such as those occurring in the nervous system that rely on interactions between different types of cells. However, although transgenic mice models of AxD showed GFAP aggregation, they could not reproduce neurological abnormalities or white matter degeneration and they were not effective in identifying the pathogenetic mechanisms induced by *GFAP* mutations nor in drug screening. Zebrafish is a teleost commonly adopted for studying nervous system development and to set up vertebrate models of monogenic neurodegenerative diseases. Due to transparency during embryogenesis and early life, zebrafish provides a very suitable model system to observe the early onset development of the disease. Therefore, the aim of this study has been the production of a zebrafish model for AxD, in order to have a system more complex than cell cultures and more reliable than mice models, suitable to perform drug screening. To this aim, we have used an approach based on Tol2 transposon, a system used to express exogen proteins in zebrafish. Zebrafish embryos were microinjected at 1 cell stage with pTol2-pgfap-GFAP WT-GFP and pTol2-pgfap-GFAP (R239C)-GFP plasmids encoding wild type or mutant GFAP fused to GFP, whose expression in glial cells was driven by the promoter of the zebrafish *gfap* gene. Embryos expressing GFAP-GFP have then undergone functional validation to assess several features already observed both in in vitro and other in vivo models of AxD. We confirmed the glial localization of aggregates by immunocytochemistry and electron microscopy, more frequent in cells expressing mutant p.R239C than WT GFAP. Besides, our results showed the positive effects of ceftriaxone treatments and sHSPs stimulation on mutant embryos p.R239C, both in terms of GFAP aggregates reduction. Our results globally support the use of zebrafish as a powerful model for both the study of the molecular pathogenesis of AxD and likely suitabel for high-throughput drug screenings for AxD specific therapies.

Acknowledgement

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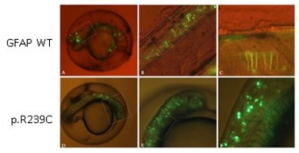
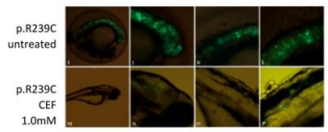


GFP expression in transgenic GFAP WT and GFAP R239C embryos
(A), (B) The expression of GFAP WT-GFP protein is visible along the neural tube, with very few GFAP aggregates observed. (C) High magnification of embryo in (B) shows GFAP WT protein as filamentous structures. (D), (E), (F) Several aggregates of p.R239C in the brain and along the neural tube are found. Magnification: 4X (A), (D); 10X (B), (E); 20X (C), (F) (from Candiani et al, 2020).



Effects of 48 hours CEF 1.0 mM treatment on embryos expressing GFAP R239C-GFP.
Images (I) to (L) show aggregates at 24 hpf (t0), while figures (M) to (P) show aggregates at 72 hpf (t48), after 48 hours of treatment with CEF 1.0 mM. (part of Fig 6, Candiani et al, 2020).

T05-007A

Early changes in cortical glia in SOD1^{G93A} mouse

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The neurodegeneration of upper motor neurons in the brain cortex, brain stem and lower motor neurons in the spinal cord is characteristic for patients with Amyotrophic lateral sclerosis (ALS). Therefore, majority of previous studies focused mostly on motor neurons, trying to explain the mechanism of their degeneration. Later on, the supporting cells, glia, became known as contributors to the disease, but their individual roles have not been elucidated yet. Here, we aimed to clarify the ongoing processes in astrocytes, microglia and oligodendrocytes during the progression of ALS. We focused on pathology in the brain, specifically in the sensorimotor cortex and tried to unveil early changes in the above-mentioned glia. We used an animal model expressing the G93A mutant form of human superoxide dismutase 1 (SOD1) and conducted two types of sensorimotor testing (modified wire grid hang test and Rotarod) to detect symptom onset and observe disease progression. Based on the results we set P30 as a presymptomatic phase, P60 as a disease onset point when first significant differences started to show, and P90 as a symptomatic phase with significant differences in performance between mutant animals and their age-matching controls. In mutants, we observed a slightly delayed onset of symptoms in females. To recognize changes in the expression profiles we employed single-cell RNA-sequencing of cortical astrocytes, microglia and oligodendrocytes from P30, P60 and P90 animals. We used both males and females to see differences in the progression between genders. Analysis of differential expression in mutant glia in P30 showed predominantly deregulation of genes associated with the electron transport chain and misfolded proteins, due to mutant SOD1. In P60 and P90, genes associated e.g. with astrocytic and microglial activation, phagosome, neuroinflammation or lipid metabolism were deregulated, with no significant differences between genders. We also used immunohistochemistry to see whether microglia, astrocytes adopted changes in their morphology. While there were no visible changes in astrocytic morphology in P90, microglia had shorter and twisted processes with spheroid swellings. Although the results are yet preliminary, we can conclude that in the sensorimotor cortex of $SOD1^{G93A}$ mice, the glial morphology and the gene expression profiles change as compared to control animals. These changes are probably related to early processes in glial cell and activation of pathways leading later to the neurodegeneration.

Acknowledgement

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T05-008A

Reactive astrocytes acquire deleterious and neuroprotective signatures in response to tauopathy.

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Tauopathy is a pathological hallmark of several neurodegenerative diseases including Alzheimer's disease and frontotemporal dementia (FTD)[1]. It is well described that neurodegeneration drives astrocyte reactivity [2–4] but the independent effects of tau pathology on astrocyte reactivity and the functional consequences of this response are

poorly understood. To investigate this, we utilised TRAPseq translatome analysis of astrocytes in MAPTP301S tauopathy mice. This model expresses human tau with the P301S mutation in a neuron-specific manner, driving accumulation of hyper-phosphorylated tau and leading to age-related region-specific synaptic and neuronal loss in the frontal cortex and spinal cord.

We found that neuronal tauopathy induces age-dependent gene-expression changes in astrocytes which match those found in human post-mortem disease samples. Key astrocyte signatures induced involved repression of bioenergetic and translation machinery; induction of inflammation pathways; upregulation of protein degradation/proteostasis genes, and enrichment for targets of inflammatory mediator Spi1 and the stress-activated cytoprotective Nrf2 response.

To further investigate a possible neuroprotective role of the tauopathy-induced Nrf2-mediated reactive astrocyte response, we over-expressed Nrf2 in an astrocyte-specific manner in our tauopathy mice. Astrocyte Nrf2 over-expression led to a reactive phenotype which recapitulated elements of the proteostasis signature; reduced phospho-tau accumulation; rescued brain-wide transcriptional deregulation; and delayed the onset of behavioural deficits. Thus, this work demonstrates that tauopathy induces astrocyte reactive profiles associated with both deleterious and adaptive-protective signals - the latter of which, if mimicked, can slow patho-progression.

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T05-009A

Basic astrocytic functions such as ion uptake and volume regulation are impaired in the triple transgenic model of the Alzheimer's disease

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During the progression of Alzheimer's disease (AD), astrocytes undergo morphological and functional changes that trigger pathological processes resulting in demyelination and neuronal death. These functional changes include impaired uptake of ions and neurotransmitters from the extracellular space (ECS). Changes in morphology affect the size and diffusivity of the ECS. Besides, the diffusion parameters of the extracellular space are affected by changes in the composition and structure of the extracellular matrix. In this study, we quantified astrocyte volume changes, which directly reflect changes in the function of ion and neurotransmitter uptake, during exposure to hypo-osmotic stress or high K^+ concentrations. We also quantified ECS diffusion parameters, namely ECS volume,

and tortuosity, reflecting both changes in cell volume and structural modifications in the extracellular matrix. These changes were monitored during aging, which is a physiological neurodegenerative process, and during the progression of AD. Additionally, we employed single-cell RT-qPCR to identify possible alterations in the astrocytic expression of transport proteins that are involved in the uptake of ions and neurotransmitters. The experiments were performed in the triple transgenic model of Alzheimer's disease (3xTg-AD), which contains three mutations associated with familial Alzheimer's disease. Based on the cell volume quantification we suggest that the ability of ion uptake decreases with age and that this phenomenon is manifested earlier in 3xTg-AD mice. Interestingly, we observed that the astrocyte volume did not change uniformly and the number of cells that swell slightly or do not swell at all increases in 3xTg-AD mice. Single-cell RT-qPCR also revealed two distinct groups of cells with higher and lower expression of homeostatic genes, which can correlate with the two groups divided based on volume changes. Quantification of the ECS diffusion parameters showed increasing tortuosity during aging and in 3xTg-AD mice, reflecting probably the accumulation of amyloid plaques as well as other structural changes of the extracellular matrix. Elucidating the existence and function of different astrocyte populations in the brain tissue affected by Alzheimer's disease should be an important step in the development of therapeutic approaches to neurodegenerative diseases.

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T05-010A

Endogenous expression of HERV-W envelope protein leads to impaired glial functions in a mouse model of multiple sclerosis

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Axonal degeneration is central to clinical disability and disease progression in multiple sclerosis (MS). Myeloid cells such as brain resident microglia and blood-borne monocytes are thought to be critically involved in this degenerative process. However, the exact underlying mechanisms have still not been clarified. We have previously demonstrated that human endogenous retrovirus type W (HERV-W) negatively affects oligodendroglial precursor cell (OPC) differentiation and remyelination via its envelope protein pathogenic HERV-W (HERV-W) ENV (formerly MS-associated retrovirus [MSRV]- ENV). In a current study, we investigated whether HERV-W ENV also plays a role in axonal injury in MS. We found that in MS lesions, HERV-W ENV is present in myeloid cells associated with axons. Focusing on progressive disease stages, we could then demonstrate using an ex vivo myelination model that HERV-W ENV induces a degenerative phenotype in microglial cells, driving them toward a close spatial association with myelinated axons. Moreover, in HERV-W ENV-stimulated microglia were found to structurally damage myelinated axons. Taken together, our data suggest that HERV-W ENV-mediated microglial polarization contributes to neurodegeneration in MS (published in Kremer, Gruchot et al., PNAS (2019); https://doi.org/10.1073/pnas.1901283116). For functional validation in vivo, we currently analyse neuronal- and glial cell responses, remyelination and neurodegeneration processes following cuprizone mediated demyelination in a novel HERV-W ENV expressing transgenic mouse model, the current findings of which will be presented here. Thus, this analysis provides a neurobiological rationale for a recently completed clinical study in MS patients showing that antibody mediated neutralization of HERV-W ENV exerts neuroprotective effects.

T05-011A

Fibroblast growth factor 9 (FGF9) mediates neurodegeneration in the adult nervous system.

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Fibroblast growth factor (FGF) signalling is dysregulated in many neurological and psychiatric conditions but there is no consensus as to its pathophysiological significance. Recent studies demonstrated FGF9 is not only up regulated in affected brain regions in major depressive disorder (MMD), but also at sites of ongoing tissue damage in multiple sclerosis (MS); an inflammatory demyelinating disease in which depression is a major co-morbidity; observations that led us to explore how FGF9-dependent mechanisms contribute to these diseases.

We report FGF9 rapidly compromises the function and survival of post-mitotic cortical neurons *in vitro.* Multi-electrode arrays revealed FGF9 rapidly blocked development of light-driven neuronal activity, an effect associated with subsequent loss of $Map2^+$ neurons and a corresponding decrease in neurite density. These effects were preceded by down-regulation of neuronal gene networks required to maintain synaptic function; an important pathogenic substrate in MS, MDD and many other neurodegenerative and neuropsychiatric diseases. The physiological significance of these observations was confirmed by using an adeno-associated viral vector to over-express FGF9 in the rat cortex. This confirmed FGF9 rapidly induces neuronal death *in vivo* resulting inneurodegeneration, secondary demyelination and ultimately lesions that replicate the gross pathology of chronic grey matter lesions in MS. Namely loss of neurites and neurons associated with recruitment/activation of microglia/macrophages and astrocytosis in the absence of large numbers of $CD3^+$ T cells recruited from the periphery.

Our data demonstrate FGF9 can contribute to the pathogenesis of MS and MDD by virtue of its detrimental effects on neuronal function and survival, an observation identifying FGF9-dependent pathways in neurons as a novel therapeutic target to slow if not halt brain atrophy in these diseases.

T05-012A

Astrocyte-specific alterations upon aging and Alzheimer's disease.

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The mechanisms leading to pathology in aging-related neurodegeneration, including Alzheimer's disease (AD), remain incompletely understood. Astrocytes have been shown to play a crucial role in the brain but their exact role in neuronal protection and survival during neurodegeneration remains unclear. Here we aim to assess how two molecules involved in autophagy (TFEB) and cell adhesion, communication and inflammation (IGTB3) are impacted by AD pathology specifically in astrocytes. 5xFAD mice and their wild-type littermates were used at two

ages, 6-7 and 11-12 months. Behavioral testing demonstrated the presence of cognitive impairment in the 5xFAD mice at the age of 6 months. Immunocytochemical and biochemical assays revealed both age- and genotype-depended alterations in TFEB and IGTB3 protein levels in adult astrocytes harvested from the mouse brains and grown in vitro. Furthermore, histological stainings of 5xFAD mouse brains revealed region-specific changes in these targets in GFAP-positive astrocytes. Taken together, the observed alterations of astrocytic TFEB and IGTB3 suggest the involvement of these molecules in astrocyte-associated compensatory mechanisms during aging and AD. These targets could serve as potent candidates for astrocyte-specific therapeutic modulation.

T05-013A

EphB3 and Deleted in Colorectal Cancer (DCC) dependence receptors contribute to oligodendrocyte cell death after brain injury

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Over 65 million people worldwide suffer from a traumatic brain injury (TBI) annually (Dewan, *et al*., 2019). Despite several clinical trials attempting to prevent cell loss after injury, therapeutic strategies have been underwhelming. This may be partly due to a lack of understanding of cell death signaling mechanisms. In contrast to classical receptor activation, dependence receptor signaling occurs when its ligand is absent. We are the first to identify that dependence receptor activation can also initiate pro-apoptotic signaling after injury, providing new insight as to how cell death propagates for several days after brain injury. We recently showed how ephrinB3 ligand displacement leads to EphB3 receptor cleavage, recruitment of Caspase 8/9 and Dral/FHL-2, resulting in oligodendrocyte cell death in the injured brain (Tsenkina and Tapanes, *et al*., 2020). We anticipated that there are additional classes of dependence receptors that contribute to neuronal and glial cell loss after injury. Thus, we evaluated how another dependence receptor, deleted in colorectal cancer (DCC) receptor and its Netrin-1 ligand, may also contribute to oligodendrocyte cell death after a moderate brain injury. We hypothesized that blocking DCC's pro-apoptotic cleavage will lead to greater cell survival and functional recovery after injury. Indeed, we show that DCC receptor signaling contributes to oligodendrocyte cell death in a murine controlled cortical impact injury model, as well as affecting myelin integrity and behavioural motor recovery. In short, our findings identify a novel mechanism of oligodendrocyte cell death in the traumatically injured brain that may reflect an important neuroprotective strategy.

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T05-014A

Dangerous Air: How Air Pollution Affects Astrocyte Functions.

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Outdoor air pollution is the largest environmental risk factor that has been associated with cardiovascular, lung, and lately also neurodegenerative diseases. A few studies have been conducted to demonstrate that air pollutant exposure may lead to neuroinflammation, oxidative stress and the appearance of protein aggregates in the brain. It was shown that air pollution can cause not only peripheral effects, but airborne particles can also enter the brain directly through olfactory nerve road or enter the blood circulation. Therefore, there is an unmet need for understanding how different brain cell types are involved in this pathological process. In this study, we aim to decipher how size-segregated urban particulate matter (PM) that was collected from urban air in Nanjing, China affects astrocytes and their functions and to investigate genes that could be targeted to mitigate the adverse effect of PM exposure. Our results demonstrate that both ultrafine (particles with an aerodynamic diameter of 0.1 μm or less) and coarse (size 2.5-10 μm) particles trigger activation of antioxidative stress signalling genes *in vitro* in astrocytes harvested from the adult mouse brain, indicating activation of the cellular protection system in response to PM. Such robust effects were not observed in primary cortical neurons exposed to the same conditions, indicating the predominant role of glial cell responses to adverse effects of air pollution in the brain. We also detected reduced *ApoE* expression in both adult and neonatal PM-treated astrocytes in response to PM exposure. These results provide insight into astrocyte responses to air pollutant exposure.

T05-015B

The contribution of glia to the onset of Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline and the deposition of amyloid beta plaques and tau tangles. Activated glia cells, such as astrocytes and microglia, surround the amyloid plaques. This activation of glia cells, called gliosis, might be neuroprotective in removal of the plaques, while also detrimental by the release of cytokines by both types of glia damaging neuronal structures. The aim of this study is to determine whether gliosis contributes to early cognitive decline in AD, already before the presence of plaques. The commonly used AD mouse model APPswe/PS1dE9 was used to study the disease. Specific markers of astrocytes and microglia, earlier identified as altered during gliosis, were used to study the onset of gliosis. We

found that gliosis is present in young AD-mice before the presence of plaques. These experiments will be followed by pharmacological interference with gliosis to study how this affects cognitive behaviour. Consequently, proteomics by mass spectrometry will be used to identify altered expression patterns of proteins that could identify the underlying molecular mechanisms. Taken together, this approach will provide information about early onset gliosis in AD and, ultimately, how early stage interference might be beneficial in treating AD.

T05-016B

Loss of SARM1 does not protect against axonal degeneration in a late-onset CMT1B mouse model.

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In most CMT1 neuropathies, demyelination precedes axonal loss, which can be confounding when attempting to specifically address the mechanisms of axonal degeneration. Remarkably, the substitution of Threonine 124 by a Methionine in the myelin protein zero (*MPZ)* gene (P0T124M) results in an axonal neuropathy (late onset CMT1B or CMT2J) with little to no myelin damage, suggesting that the two processes are separable. To investigate axonal degeneration mechanisms in CMT1, we generated the P0T124M mouse model. P0T124M mice fully recapitulate axonopathy observed in patient and allow us to test therapeutic approaches to limit axon loss. One of the most promising “druggable” target to counteract axonal degeneration is SARM1 (sterile alpha and toll/interleukin receptor motif-containing 1). By its NADase activity, SARM1 is believed to be the central executioner of the axonal degenerative program. The decrease of NAD+ levels and the increase of plasmatic NF-L in P0T124M mice suggest that SARM1 could be involved in axon loss in CMT2J. To test the role of SARM1 in our model, we crossbred P0T124M mice with SARM1-/- mice. We determined the consequences of SARM1 deletion to axonal damages using electrophysiological measurements, electron microscopy and genetic tools.The analysis of 12-month-old P0T124M//SARM1-/- mice, shows that SARM1 deletion does not rescue the axonopathy observed in P0T124M mice. As P0T124M mutants, P0T124M//SARM1-/- mice present a reduction of compound motor action potential amplitudes and nerve conduction velocities, as well as reduction of myelinated fibers and an increase of degenerative axons. Finally, using a Thy-YFP reporter line, we identified tips of degenerating axons (axonal swelling and fragmentation) in P0T124M//SARM1-/- nerves. SARM1 deletion does not confer long-term protection from axonal degeneration in a late-onset CMT1B model. Now, it will be crucial to determine if SARM1 deletion could represent a valuable therapeutic target to limit axonal loss in early-onset CMT disease.

T05-017B

The potential role of the neuroinflammatory response in Purkinje cell damage: a prion disease as natural model of neurodegenerative diseases

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The crucial role of the immune response of the brain in neurodegenerative disorders, as described by the current neuroinflammatory hypothesis, is being increasingly acknowledged. The real involvement of neuroglial cells in this process, on the basis of their capability to react against central nervous system damage, but also of their possible involvement in the mechanisms contributing to neurodegeneration, is being widely studied in last decades.
Variations in markers potentially related to inflammation throughout natural Scrapie progression by using sheep cerebella at different clinical stages (preclinical, clinical and terminal) are comparatively assessed to healthy individuals. The final goal consists of determining the role of the host immune system playing in the neurodegenerative progress. To provide a global profile of the neuroinflammatory process in prion diseases is specifically expected here paying particular attention to Purkinje cells as most injured neurons in this brain area from naturally Scrapie affected animals and their interaction with glial cells.
The results provided here suggest a relevant immunological component in the progression of damage of those neurons studied in this model of natural neurodegeneration.
It results relevant to be reasserted the extremely high value of using natural models to provide reliable results. Taking into account that transgenic models have demonstrated poor representativeness of natural disease progress, natural models used as here constitutes a powerful *in vivo* tool for approaching how infection occurs. To determine the meaning of all these alterations in the process of neurodegeneration is essential to advance in knowledge about pathogenesis of these pathologies.

T05-018B

Hemin-mediated oxidative damage in the central nervous system selectively targets myelinated axons

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There is mounting evidence that localized accumulation of iron derived from haemoglobin breakdown products exacerbates oxidative injury in multiple sclerosis, but their cellular specificity and mode of action remain unclear. To explore this in more detail we focused on hemin (ferriprotoporphyrin IX), a major iron-containing haemoglobin breakdown product, and investigated its effects in dissociated spinal cord cultures that replicate the cellular complexity and functional properties of the CNS.
We demonstrate hemin mediates a dose-dependent cytotoxic effect that preferentially targets oligodendrocytes and myelinated axons in these cultures, but spares $NeuN^+$ neurons. Its cytotoxic potential is iron dependent and > 25-fold greater than equimolar concentrations of Fe^{3+} indicating its porphyrin-ring potentiates its cytotoxic effect; an interpretation supported by experiments demonstrating protoporphyrin IX, and its Zn^{2+} adduct, are not cytotoxic in these cultures. A series of studies using selective inhibitors to target specific pathways reveals hemin-mediated cytotoxicity in this culture system involves contributions from reactive oxygen species and ferroptosis, but is

independent of hemin/iron-mediated microglial activation. Our data suggest hemin rapidly partitions into the hydrophobic environment of the oligodendrocyte/myelin continuum resulting in localised lipid and membrane protein destabilisation and, ultimately, cell death.
Gaining insights into how hemin facilitates demyelination/ axonal degeneration will help identify new strategies to limit tissue damage and disease progression in MS and haemorrhagic stroke.

T05-019B

Reactive astrocytes acquire beneficial anti-aggregation properties through the JAK2-STAT3 pathway in Huntington's disease

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Huntington's disease (HD) is an inherited neurodegenerative disease caused by the mutation of the Huntingtin (HTT) gene. HD is characterized by motor, cognitive and psychiatric alterations and the presence of intracellular aggregates of mutant huntingtin (mHtt). The degeneration of specific neuronal populations in the striatum and cortex is the main cause for clinical symptoms. However, it is now clear that astrocytes also play a role in HD pathogenesis. Astrocytes become reactive in response to virtually all pathological situations in the central nervous system. In the brain of HD patients, there is a progressive accumulation of $GFAP^+$ reactive astrocytes and transcriptomics analysis reveals significant molecular changes in HD astrocytes.
The JAK2-STAT3 pathway is a central cascade controlling the reactive response of astrocytes in a variety of diseases, including in HD mouse models. We found that STAT3 is activated in astrocytes in the putamen of stage 3 HD patients. To pinpoint the role of reactive astrocytes in HD, we generated viral vectors that specifically infect astrocytes in the mouse striatum and activate or inhibit the JAK2-STAT3 pathway in situ. We show that JAK2-STAT3-controlled reactive astrocytes reduce the number and size of mHtt aggregates in striatal neurons in different mouse models of HD, and improved neuronal alterations. We found that the JAK2-STAT3 pathway controls the expression of proteolytic enzymes in HD astrocytes (see also poster "Riquelme-Pérez. et al."), enhances proteosomal and lysosomal activities in HD astrocytes and increase astrocyte capacity to clear mHtt. We also found that JAK2-STAT3-dependent reactive astrocytes produce chaperones, which are released in exosomes and could be taken up by neurons to promote the clearance of mHtt.
Overall, our data show that reactive astrocytes acquire protective properties in HD, through the JAK2-STAT3 pathway, with enhanced proteolysis capacity and anti-aggregation signaling with neurons. We show that astrocytes are not only dysfunctional cells in HD, as usually reported, but instead can engage in protective reactive responses, that could be promoted for HD therapy.

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T05-020B

Crosstalk between human iPSC-derived astrocytes and neurons in a cell culture model of Alzheimer's disease

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The way in which Alzheimer's disease (AD) spreads in the brain has been debated for many years. Experimental evidence indicates that amyloid β (Aβ) aggregates can indeed seed and transmit pathology throughout the tissue. Several possible transfer mechanisms have been proposed, including cell-to-cell transmission and extracellular vesicle (EV) secretion. The aim of this study is to investigate the crosstalk between human astrocytes with inclusions of amyloid β and human neurons. Previous results from our laboratory demonstrate that mouse astrocytes with intracellular Aβ deposits secrete EVs with partially digested Aβ peptides that induce neurodegeneration and apoptosis of primary cortical neurons (1,2). Following up on those results, we are investigating if human astrocytes with intracellular Aβ deposits affect human neurons in a similar fashion, by spreading toxic Aβ species via EVs or direct cell to cell interaction.

For this purpose, we have set up a co-culture system of astrocytes and neurons generated from human induced pluripotent stem cells (hiPSCs). Shortly, hiPSCs are differentiated to neuroepithelial stem (NES) cells, and then further differentiated to mature astrocytes and neurons, under strictly defined protocols (3,4). The fully differentiated astrocytes are exposed to sonicated Aβ42 fibrils for three to six days. Then the cultures are carefully washed and cultured for additional time in Aβ-free medium. Conditioned medium is collected from the astrocyte cultures nine days after the wash and added to hiPSC-derived neurons. Using transmission electron microscopy (TEM) we confirmed the presence of EVs in the medium. Whether the conditioned medium induces pathology in neurons is evaluated via cell toxicity assays, TEM and immunocytochemistry, using synaptic markers and markers for phosphorylated tau. In parallel to the conditioned medium-experiments we are developing co-cultures of Aβ-exposed astrocytes and neurons in order to examine any effects that their direct contact may have on neuron survival. By using using labelled Aβ42 fibrils we can also track transmission mechanisms between astrocytes and neurons, including tunneling nanotube transfer or membrane fusion, using timelapse microscopy.

Taken together, our data from this study will contribute with important information about how the neuron-astrocyte interplay may affect AD phenotype propagation.

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T05-021B

Selective vulnerability of inhibitory networks in multiple sclerosis

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In multiple sclerosis (MS), a chronic demyelinating disease of the central nervous system, neurodegeneration is detected early in the disease course and is associated with the long-term disability of patients. Neurodegeneration is linked to both inflammation and demyelination, but its exact cause remains unknown. This gap in knowledge contributes to the current lack of treatments for the neurodegenerative phase of MS. Here we ask if neurodegeneration in MS affects specific neuronal components and if it is the result of demyelination. Neuropathological examination of secondary progressive MS motor cortices revealed a selective vulnerability of myelinated inhibitory interneurons in MS. The generation of a rodent model of focal subpial cortical demyelination reproduces this selective neurodegeneration with the specific reduction in parvalbumin-expressing interneurons, loss of inhibitory synapses and inhibitory post-synaptic currents after demyelination. In contrast, remyelination coincides with an increase in inhibitory synapses despite the loss of neurons and axons in the area. Our results show that there is a selective neurodegeneration, secondary to demyelination in MS and provide a new preclinical model for the study of neuroprotective treatments.

T05-022B

Manipulations of GSH content modulate $[Ca^{2+}]_i$ homeostasis in rat astroglioma cells

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Introduction: Oxidative stress is an aggravating factor of all neurological disorders, including neurodegenerative diseases. It is due to an exaggerated accumulation of reactive oxygen species (ROS) concomitant to decreased reduced glutathione (GSH) levels; the main antioxidant of brain cells produced by astrocytes in large amounts. Cell death triggered by oxidative damage results from intracellular calcium overload which is deleterious to cell activity. The mechanisms involved in these calcium increases are not fully understood. Here, we have thus investigated whether GSH could protect neurons against oxidative stress through the modulation of calcium homeostasis. For this, GSH cell content was manipulated by modulating its metabolism.

MM and results: To investigate the link between GSH content and calcium homeostasis, we pre-treated rat astroglioma cells (C6) with sulforaphane (SFN) to increase intracellular GSH levels, or buthionine sulfoximine (BSO) to decrease them, during 24 hours for both treatments.
We show using calcium imaging and electrophysiological “patch-clamp” recordings that increasing GSH levels reduced the intracellular mobilization from IP3-sensitive intracellular calcium stores triggered by P2Y purinergic receptor stimulation and the associated membrane conductance changes.
By contrast, depleting GSH cellular content by impeding its synthesis with BSO, resulted in a sensitization of Calcium Release Activated Channels (CRAC) evidenced by GSK-7975A, a CRAC blocker. Indeed, in BSO-treated cells, firstly, Ca2+ increases via CRAC were observed under oxidative stress and, secondly, store-operated calcium entry (SOCE) was revealed after P2Y receptor activation.
According to this, quantitative PCR and Western Blotting revealed modified expression of the proteins involved in SOCE, i.e. Orai and STIM, when cells were treated with either SFN or BSO.
Conclusion: We conclude that GSH cell content changes impact intracellular Ca^{2+} homeostasis in C6 glioma cells likely via sensitization/desensitization mechanisms of Calcium Release Activated Channels (CRAC). GSH depletion due to oxidative stress regulation could thus contribute to the alterations of Ca^{2+} homeostasis in astrocytes under pathological conditions.

T05-023B

ALS reactive astrocytes impair neuromuscular junctions in microfluidic devices

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disorder in adults. The disease affects both upper and lower motor neurons resulting in muscle atrophy, paralysis and death of patients usually within 2 to 5 years after symptom onset. Although ALS primarily causes motor neuron death, astrocytes clearly contribute to the disease as well. Activation of this cell type is a prominent hallmark in ALS, and the number of reactive astrocytes correlates with disease progression. Despite the growing research interest in the astroglial contributions, little is known about their exact role and means of toxicity.

In this study, we cultured ALS-patient iPSC-derived motor neurons and astrocytes together with healthy human myoprogenitor-derived myotubes in microfluidic devices. This model allowed us to investigate the mechanisms of neuromuscular junction (NMJ) pathology, which is considered crucial in early ALS progression, as well as studying the role of astrocytes. We discovered a patient-specific reduction in neurite outgrowth and an impairment of NMJ formation, which was astrocyte dependent. Mutant astrocytes displayed a toxic phenotype towards both mutant and isogenic motor neurons and NMJs, while isogenic control astrocytes rescued these aberrations. We could also demonstrate that ALS astrocytes displayed increased reactivity and emission of pro-inflammatory cytokines in comparison to their isogenic control counterparts. Altogether, we established a new model allowing us to investigate the astroglial importance in ALS disease, which provides us with better insights into the underlying mechanisms of this neurodegenerative disorder.

T05-024B

Understanding the nature of astrocyte reactivity in acute perinatal white matter injury and role in disease pathogenesis

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Introduction

White matter injury (WMI) is the most common form of brain injury in preterm infants and a major cause of long-term neurological morbidity. It is characterized by reactive microgliosis and astrocytosis, and defective oligodendrocyte maturation. Recent studies in the injured mature brain show the formation of diverse reactive astrocyte subtypes with contrasting roles after injury, some favoring brain repair and other «inflammatory» astrocytes contributing to neurodegeneration. The specific nature of astrocyte reactivity after WMI remains obscure. We know that these inflammatory astrocytes are induced by activated microglia-derived IL-1α, TNF, and C1q and lead to myelination failure, an outcome characteristic of perinatal WMI. Given that inflammatory astrocytes inhibit the maturation of myelinating cells, we hypothesize that inflammatory astrocytes play a central role in WMI and may be an exciting therapeutic target for this disease. We report the results of experiments aimed to investigate the formation, function and therapeutic modulation of inflammatory astrocyte (iAs) in WMI.

Materials and Methods

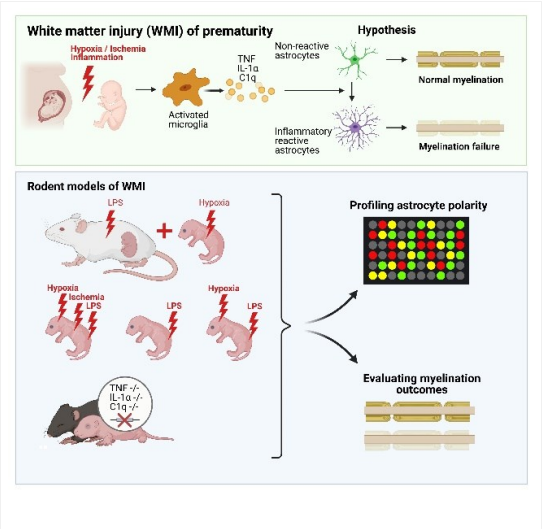
We tested the formation of iAs across multiple rodent WMI models using a combination of hypoxic-ischemic and inflammatory insults. To confirm experimental WMI, myelin deficits were evaluated using immunohistochemistry (IHC) for myelin basic protein (MBP) at postnatal day 11. iAs formation was investigated through in situ hybridization (ISH) using a complement protein 3 (C3)-specific probe. We further characterized astrocyte reactivity by performing microfluidic qRT-PCR analysis using a panel of known reactive astrocyte transcripts on mRNA isolated from primaryastrocytes purified through immunopanning from injured and healthy brains. IL-1α/TNF/C1q knockout mice unable to generate iAs were used to investigate the necessity of iAs for WMI outcomes.

Results

ISH demonstrates a significant increase of C3-positive iAs in subcortical white matter tracts across multiple rodent WMI models. Supporting this finding, preliminary qRT-PCR results suggest that purified primary astrocytes from injured brains exhibit a multi-gene inflammatory astrocyte signature at the transcriptome level. Ongoing experiments in mutant mice test whether iAs are central drivers of WMI pathogenesis.

Conclusion

Our experiments demonstrate the formation of inflammatory reactive astrocytes in multiple rodent models of WMI, test these cells' ability to drive WMI outcomes, and work towards an in-depth characterization of astrocyte polarity in this disease. This result is an important step towards understanding astrocyte polarization in WMI and opens the door to experiments investigating whether preventing the formation of this astrocyte subtype ameliorates WMI disease outcomes.



What is the role of reactive astrocytes in perinatal white matter injury?

T05-026B

Human iPSC-derived astrocytes transplanted into the mouse brain display differential responses to amyloid-β

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Background: Increasing evidence for a direct contribution of astrocytes to neuroinflammatory and neurodegenerative processes causing Alzheimer's disease comes from molecular studies in rodent models. However, these models may not fully recapitulate human disease as human and rodent astrocytes differ considerably in morphology, functionality, and gene expression.
Methods: To address these challenges, we established an approach to study human astroglia within the context of the mouse brain by transplanting human induced pluripotent stem cell (hiPSC)-derived glia progenitors into neonatal brains of immunodeficient mice.
Results: Xenografted (hiPSC)-derived glia progenitors differentiate into astrocytes that integrate functionally within the mouse host brain and mature in a cell-autonomous way retaining human-specific morphologies, unique features and physiological properties. In Alzheimer's chimeric brains, transplanted hiPSC-derived astrocytes respond to the presence of amyloid plaques with various morphological changes that seem independent of the *APOE* allelic background.
Conclusion: In sum, this chimeric model has great potential to analyze the role of patient-derived and genetically modified astroglia in Alzheimer's disease.

T05-027B

Retinal changes in the APP$^{NL-F/NL-F}$ mouse model: a SD-OCT study from 6 months to 20 months of age.

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Purpose: In Alzheimer's disease (AD) even before changes appear in the brain, presents retinal neurodegeneration, suggesting the retina as an accessible biomarker of AD. This research is a diachronic study using spectral domain optical coherence tomography (SD-OCT) to measure the total retinal thickness and retinal nerve fiber layer (RNFL) thickness at 6, 9, 12, 15, 17, and 20 months old, in an $APP^{NL-F/NL-F}$ mouse model of AD compared to wild type (WT) animals.

Methods: Total retinal thickness and RNFL thickness were determined in $APP^{NL-F/NL-F}$(n=55) and WT (n=41) animals. The mean total retinal thickness was analyzed following the Early Treatment Diabetic Retinopathy Study sectors. RNFL was measured in six sectors of axonal ring scans around the optic nerve. Microglial and astroglial activation was evaluated at 17-months-old, by analyzing vertical retinal sections immunostained with Iba-1 and GFAP.

Results: In the $APP^{NL-F/NL-F}$group compared to WT animals, for total retinal thickness was observed: i) At 6-months-old, a significant thinning in the outer temporal sector; ii) at 15-months-old a significant thinning in the inner temporal and in the inner and outer inferior retinal sectors; iii) at 17-months-old, a significant thickening in the inferior and nasal sectors in both inner and outer rings; and iv) at 20-months-old, a significant thinning in the inner ring of nasal, temporal and inferior retina and in the outer ring of superior and temporal retina. In RNFL thickness, a significant thinning in the global analysis and in nasal and inner-temporal sectors at 6 months old were observed. In addition, in the $APP^{NL-F/NL-F}$group we found activation of microglial cells (thicker somas and processes and amoeboid forms) and astroglial cells (higher GFAP+ immunostaining with astrocyte clusters in certain areas).

Conclusions: In the $APP^{NL-F/NL-F}$ AD model, the retinal thickness showed thinning, possibly produced by neurodegeneration alternating with thickening caused by deposits and neuroinflammation in some areas of the retina. These changes over time are similar to those observed in the human retina and could be a biomarker for AD. The $APP^{NL-F/NL-F}$ AD model may help us better understand the different retinal changes during the progression of AD.

T05-028B

Mild aging phenotype of microglia, astrocytes and neurons in CD1 mice

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Microglia are tissue-resident immune cells of the central nervous system and are strikingly involved in the maintenance of tissue homeostasis and surveillance of the brain. Microglia are characterized by their highly ramified morphology and equal distribution in the brain tissue, which is a prerequisite to exert their function. Using custom-written, three-dimensional image analysis tools, region-specific differences in microglia morphology were identified in cortex, hippocampus, striatum and hypothalamus. Previous studies have reported microglial alterations in the course of aging in C57BL/6 mice similar to disease-induced activation. Age-related alterations of microglial cell number, morphology and distribution were assessed in Iba1-immunolabeled tissue sections from adult (6 month-old) and aged (24 month-old) CD1 mice. Interestingly, the morphology of microglia in aged CD1 mice resembled the microglial phenotype found in young CD1 mice. Furthermore, aging is associated with changes of neurons and astrocytes. In this regard, neuronal density was analyzed in substantia nigra and in the hippocampus -

regions prone to the process of aging. Similarly, no age-related neuronal loss was found. To investigate astroglial changes during aging, astrocytic cell density, activation level in terms of GFAP fluorescence and cell morphology were quantified in different brain regions. Accordingly, adult astrocytic phenotype was preserved in aged CD1 mice. Moreover, histological analysis was complemented by transcriptomic profiling of adult and aged FACS-sorted microglia. Interestingly, no loss of homeostatic gene expression was detected in aged microglia. In summary, region-specific types of microglial morphology were identified using automatic image analysis tools and assessed in the course of aging. Mild aging phenotype in microglia and astrocytes and the absence of neuronal loss were observed in aging CD1 mice. This inquiry helps to unravel age-related changes that affect major cell types in the brain and constitute a major risk factor for neurodegenerative diseases.

T05-029C

The partial deletion of mGluR5 affects the pro- and anti-inflammatory and bioenergetic characteristics of microglia during ALS progression in SOD1^{G93A} mice

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Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disease characterized by death of upper and lower motor neurons (MNs). Even if the etiology is not completely understood, it is known that ALS is a multifactorial disease in which several mechanisms concur to disease progression, being Glutamate (Glu)-mediated excitotoxicity one major cause. ALS is also a non-cell autonomous disease, since glial cells, in addition to motor neurons, contribute to the pathology progression, acquiring a reactive phenotype. Several evidence support the role of Group I metabotropic glutamate receptors, which comprise mGluR1 and mGluR5, in ALS. In particular, mGluR5 are deeply involved in disease shaping, even if their importance in the different neural cellular populations has not been thoroughly investigated. In our previous studies we generated double mutant mice carrying the ALS-specific SOD1^{G93A} mutation and the mGluR5 partial deletion (SOD1^{G93A}mGluR5$^{+/}$). These mice showed increased survival, amelioration of disease progression and of its cellular and molecular features.

The aim of this study was to investigate the effect of the in-vivo genetic partial deletion of mGluR5 in SOD1^{G93A} mice on microglial cells. Microglial cells were acutely isolated by a discontinuos Percoll gradient from motor cortex and spinal cord of WT, SOD1^{G93A} and SOD1^{G93A}mGluR5$^{+/-}$ mice at pre, early- and late -symptomatic stages of ALS. TMEM119-positive microglial cells derived from the different animal groups were analyzed by flow cytometry and the balance between the M1-like pro-inflammatory and M2-like anti-inflammatory microglia phenotype was studied by using a panel of specific markers. mGluR5 expression and localization was analyzed by confocal microscopy and the oxygen consumption and ATP synthesis were evaluated by oximetric and luminometric analysis. In addition, the enzyme activities involved in aerobic and anaerobic glucose metabolism were studied.

The M1/M2 ratio augmented in the spinal cord of SOD1^{G93A}, and in SOD1^{G93A}mGluR5$^{+/-}$ mice at the late symptomatic phase of the disease in spinal cord-derived microglia, while no significant changes were observed at the other stages of the disease and in motor cortex. The bioenergetic data highlighted alterations of oxygen consumption and ATP synthesis, that are partially restored in SOD1^{G93A}mGluR5$^{+/-}$ mice.

Our data demonstrate that the reduction of mGluR5 in SOD1^{G93A} mice forces the spinal cord-derived microglia toward a more pro-inflammatory phenotype while restore the energetic metabolism failure.

T05-030C

Aberrant expression of the axon guidance cue netrin-1 by SOD1-ALS astrocytes contributes to motor neuron degeneration.

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Mutations in the ubiquitously expressed superoxide dismutase-1 (SOD1) gene cause ~20% of inherited amyotrophic lateral sclerosis (ALS). The underlying molecular mechanisms in mutant SOD1 linked ALS involve a combination of direct motor neuron and glia-induced toxicity. Although astrocytic contribution towards motor neuron death in ALS is well established, the identity of the mediators of this toxicity is not well understood. We previously discovered in the SOD1-G93A mouse model of ALS that caspase-3 cleaves the astroglial glutamate transporter GLT-1 (EAAT2), releasing a sumoylated C-terminal fragment (CTE-SUMO1), which accumulates in the nucleus of astrocytes over disease and triggers non-cell autonomous motor neuron toxicity. Microarray analysis of these CTE-SUMO1 astrocytes revealed higher expression of the axon guidance cue netrin-1. Our findings are further corroborated by analyzing an interactive data exploration portal based on a recently published SOD1-ALS mouse spatial transcriptomics study. While in the adult central nervous system, only oligodendrocytes and neurons express and release netrin-1 to regulate axon homeostasis and synaptic maintenance, spinal cord astrocytes have not been shown to express netrin-1. We provide here the first evidence of aberrant netrin-1 expression in the astrocytes of the symptomatic SOD1-G93A mice. Interestingly, netrin-1 is also elevated in neurons localized to the ventral horn of the presymptomatic and disease onset stages of the SOD1-G93A mice suggesting a neuron-astrocyte cross-talk during disease progression. Elevated expression of Netrin-1 was also detected in human post-mortem ALS spinal cord astrocytes. Further, induced pluripotent stem cells (iPS) -derived astrocytes from SOD1-ALS showed a higher frequency of exocytosis and increased secretion of netrin-1. Neutralization of netrin-1 in human iPS astrocyte-conditioned media using anti-netrin-1 antibody significantly rescued the motor neuron-directed toxicity. Intrathecal injection of astrocyte-targeted netrin-1 expressing AAV5 in mice strikingly decreased the compound muscle action potential (CMAP), signifying motor dysfunction. Mechanistic studies reveal netrin-1 causes altered motor neuron excitability and increased AMPA receptor-mediated calcium signaling. Acute stimulation of mature primary motor neurons with netrin-1 (25 nM) elicits a robust Calcium influx response. Microelectrode analysis on motor neurons treated with netrin-1 showed changes in the frequency of firing both in the short term and long term. Aberrant production and release of netrin-1 by SOD1-ALS astrocytes may be one of the pathogenic mechanisms contributing to non-cell autonomous motor neuron toxicity in ALS and, therefore, be a potential novel molecular target for intervention.

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T05-031C

Using human iPSC-derived neurons and astrocytes to decipher synapse-related phenotypes in familial Parkinson's disease

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Parkinson's disease (PD) and related synucleinopathies are a group of incurable neurodegenerative disorders associated with alpha-synuclein (αSyn) pathology(1), with the best-characterized mutation, G209A, in the αSyn gene *SNCA*(2) resulting in the pathological p.A53T-αSyn protein. Although the disease mechanisms remain largely unresolved, the technological breakthrough of induced pluripotent stem cells (iPSC) provides a unique human setting for the identification and interpretation of PD phenotypes. In this context, we have created a robust iPSC-based model of PD from patients harboring the p.A53T-αSyn mutation that simulates disease-relevant phenotypes, including intraneuronal protein aggregates, compromised neuritic growth, and αSyn- and Tau-associated axonal pathology with reduced synaptic connectivity(3). Global transcriptome analysis of the p.A53T-αSyn neurons indicated defects in synapse formation and function, in line with the suggested role of αSyn in pre-synaptic events. Indeed, our latest work implementing electron microscopy on p.A53T-αSyn neurons revealed impaired organization of synaptic vesicle pools, microtubule disorganization, and a striking accumulation of autophagic vacuoles. Moreover, we used the artificial synapse formation assay to study synaptogenesis of p.A53T-αSyn neurons, and showed that although they could form synaptic-like connections, given the proper post-synaptic cue, they exhibited lower synaptic density than control neurons. Using monosynaptic rabies virus tracing we further assessed neuronal network formation in p.A53T-αSyn vis-à-vis control neurons, and detected decreased connectivity in mutant cultures. Apparently, most published work to date has focused on neuron-intrinsic dysfunction in PD while the role of other cell types, particularly astrocytes, has only started being examined(4), upon appreciation not only of their abundancy in the human brain and their critical role in maintaining neuronal health but also of their neurotoxic potential(5) that could contribute to disease pathology through neuroinflammatory mechanisms, a consistent PD feature. Given astrocyte contribution in modulating synaptic transmission, we have recently extended our research and generated midbrain-patterned astrocytes from p.A53T-αSyn patients and healthy donors using iPSC technology. Characterization of astrocytes in terms of neuroinflammatory reactivity, αSyn expression levels, and protein aggregate accumulation, is ongoing before evaluating their role in the synaptic phenotype of PD pathology. We anticipate that our model will answer fundamental questions related to PD pathogenesis and could ultimately serve as a new drug-testing platform.

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T05-032C

Galectin-3 deletion reduces acute colitis-induced pro-inflammatory microglial activation in the ventral mensecephalon

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Activation of microglial cells is an important feature of neurodegenerative diseases, including Parkinson´s disease. Under disease conditions, different microglia polarization states may be found including what has been defined as disease-associated microglia (DAM). Recent studies using single-cell transcriptomic analysis of microglia have identified modules of genes that show similar responses in multiple setting. Very interestingly, galectin-3 was found to be one the most significant upregulated genes under disease conditions, thus anticipating a major role in driving microglia-related immune responses. Recently, it has been demonstrated an intriguingly association between gut inflammation and neurodegeneration. The aim of this study is to investigate the possible beneficial effect that the genetic depletion of *Gal3* could have in the peripheral inflammation induced by an acute colitis model that resembles an active stage of UC, and the subsequent effects on brain inflammation. We believe that finding new strategies that could control both central and peripheral inflammation could be useful tools to delay the course of Parkinson´s disease.

T05-033C

CD300f immune receptor inhibition induce exacerbation of penetrating cortical injury

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Immune receptors play a critical role in regulating immune and inflammatory processes in the central nervous system (CNS). CD300f is of particular interest given its capacity to transmit activating and inhibitory signals. We previously demonstrated that the ligands of CD300f are present in the normal CNS and that the overexpression of CD300f has a neuroprotective role after an acute excitotoxic brain injury.

In order to study its biological function, we used rCD300f-Fc, a fusion protein that interrupts the endogen interaction between CD300f receptor-ligands, and CD300f KO animals, both *in vitro* as well as *in vivo*. *In vitro,* in co-cultures of hippocampal neurons and mixed glia, we observed that the fusion protein induced a dose-dependent neuronal

death. This neuronal death was dependent on glia, because no cell death was detected in enriched hippocampal neuronal cultures treated with CD300f-Fc. Moreover, we observed that conditioned medium of co-cultures of hippocampal neurons and mixed glia treated whit rCD300f-Fc induced neuronal death in enriched neuronal cultures, however conditioned medium by mixed glia treated whit rCD300f-Fc did not.

In accordance with the *in vitro* results*, in vivo* studies, performed by injecting rCD300f-Fc into rat or mouse neocortex, showed an increased lesioned areaas observed by Nissl stain or an increased neuronal loss as observed by direct counting of YFP+ neurons in Thy1-YFP-H mice. Many of the ligands of CD300f, such as phosphatidylserine, sphingomyelin or lipoproteins, are shared with TREM2 immune receptor. To address the question of whether the effects of CD300f-Fc could be mediated by the inhibition of TREM2, CD300f KO mice were injected intracortically with rCD300f-Fc or control IgG. As expected, no effect was observed by CD300f-Fc injection in CD300f KO mice when compared to control IgG injection, suggesting that the fusion protein is acting by inhibiting the endogenous CD300f receptor.

In conclusion, taken together, these data suggests that CD300f is involved in the maintenance of CNS homeostasis after an *in vivo* penetrating brain injury, and that microglial CD300f inhibition may alter microglial phenotype generating a neurotoxic microenvironment.

T05-034C

Impaired SVCT2 intracellular trafficking contributes to redox imbalance in Huntington's disease.

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Huntington's disease (HD) is a neurodegenerative disorder caused by a glutamine expansion at the first exon of the huntingtin gene. Huntingtin protein (Htt) is ubiquitously expressed and it is localized in several organelles, including endosomes. HD has been associated to a failure in energy metabolism and oxidative damage. Ascorbic acid is a powerful antioxidant highly concentrated in brain where it acts as a messenger, modulating neuronal metabolism. It is transported into neurons via the Sodium-dependent Vitamin C Transporter 2 (SVCT2). During synaptic activity ascorbic acid is released from glial reservoirs to extracellular space inducing an increase in SVCT2 localization in HD. Here, we studied SVCT2 trafficking and localization in HD. SVCT2 localization at synaptic terminals was found decreased in YAC128 mice. Using cellular models for HD (STHdhQ7 and STHdhQ111 cells), we determined that SVCT2 trafficking through secretory and endosomal pathways are altered. We observed Golgi fragmentation and SVCT2/Htt-associated protein-1 (HAP1) miss colocalization. On the other hand, SVCT2 translocation to plasma membrane is decreased in presence of extracellular ascorbic acid in HD cells. The SVCT2 delivery to cellular surface in HD cells would be impaired due to altered ascorbic acid-induced calcium signalling. Our results revealed that SVCT2 trafficking to plasma membrane is altered in HD. That should explain the redox imbalance observed in early stages of HD.

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Chilean FONDECYT grant 1151206, 1191620, 1201635 and 1201706, Chilean CONICYT grant 21110592 and International SfA grant.

T05-035C

Leukocyte infiltration: a driver of reactive gliosis?

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Retinal degenerative diseases are the leading causes of vision loss in developed countries. While treatments are evolving to manage late-stage symptoms of retinal degeneration, no effective therapies to prevent the degeneration exist. This is partly due to the multifaceted disease process that involves interplay among a variety of cellular abnormalities, triggered by local inflammation and influx of peripheral leukocytes (PL) into the retina.
The immune response to retinal insult has been studied mostly by histological examination and the lack of systems that distinguish between recruited and resident immune cells has hindered analysis of cell-type-specific functions. Here, we used a custom-built confocal scanning laser ophthalmoscope (SLO) with an embedded laser coagulator. This instrument enables to generate a well-defined laser damage to the photoreceptors under image guidance and track the dynamic response of resident microglia and infiltration of PL. *In vivo* optical assessment of induced-degenerative changes in a large neuronal field, encompassing the insult, allowed us to relate the time course of microglia and PL dynamics to that of the tissue remodeling and structural recovery. To test if PL-microglia signaling is involved in the microglial control, we reduced PL recruitment to the retina by FTY720 treatment. We showed *in vivo* that PL contribute to laser-induced degeneration in the retina, as FTY720 reduced scarring, rescued the BRB integrity. Additionally, microglia underwent morphological changes toward an amoeboid phenotype during degeneration.
Although innate immunity and its impact on retinal degenerations are widely explored, the delineation of adaptive immune milieu amongst the broad leukocyte infiltrate is poorly described in the eye. Thus, we dissected the PL into their main lymphoid subpopulation, $CD4^+$ and $CD8^+$ T-cells and analyzed *in vivo* their recruitment during degeneration in the retina. T-cell clustering in the injury at early time points (day 1-7) was associated with prolonged inflammatory response, hyper-ramified microglia, breakdown of the BRB, and scar formation (day 7-14). As FTY720 treatment affected broadly PL, we used a genetic model in which T-cells are absent systemically, Rag1 KO mice, to examine how their absence affects scar formation in response to laser-induced degeneration. Strikingly, genetic depletion of T-cells, apart from reducing the degenerating area as leukopenia, accelerated recovery of the BRB compared to FTY720-treated mice.
Collectively, we provided novel insights into the impact of T-cells on retinal degeneration. These results may ultimately contribute to the design of more effective immunotherapeutic approaches for patients with neurodegenerative diseases.

T05-036C

Investigating the role of microglia in cortical synaptic pathology in multiple sclerosis

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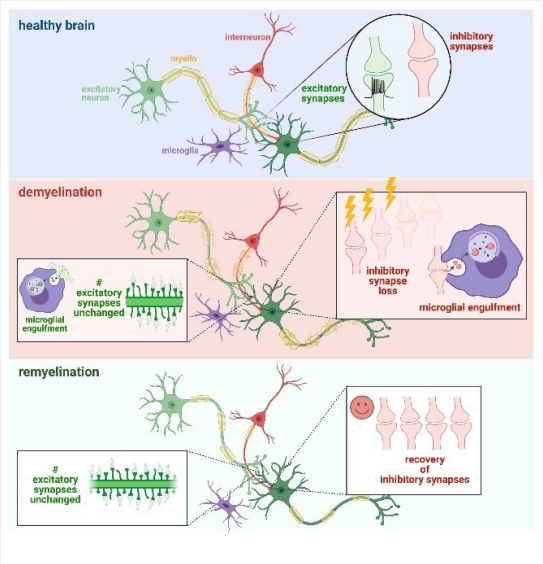
Multiple sclerosis (MS) is an inflammatory demyelinating neurodegenerative disease of the human central nervous system (CNS). MS research has traditionally focused on the white matter, but the importance of grey matter pathology is increasingly acknowledged, as neuronal loss and cortical atrophy correlate with disability progression, while MS therapeutics fail to significantly impact neurodegeneration. Evidence from other neurodegenerative diseases suggests that the structural and functional compromise of synapses may occur prior to irreversible neuronal demise, and previous work from our lab reveals a selective vulnerability of inhibitory synapses in MS grey matter. Microglia, the resident myeloid cells in the CNS parenchyma, are candidate effectors of MS-associated synaptic loss, as they are reported to eliminate synaptic connections during development and contribute to degeneration-associated synaptic compromise.

In the present study, we ask how microglia contribute to synaptic pathology in the MS grey matter using post-mortem human MS tissue, and the cuprizone mouse model of global toxin-induced demyelination followed by spontaneous remyelination. In the post-mortem cortex from donors with progressive MS, there is a specific loss of inhibitory synapses accompanied by evidence of microglial elimination of inhibitory synaptic components. The cuprizone model of cortical demyelination recapitulates this pattern of pathology, as it reveals a selective loss of inhibitory synapses early in demyelination, appearing before signs of axonal damage and in the absence of neuronal loss. Evidence of microglial engulfment of inhibitory synaptic components is present at this early stage, but not later in demyelination, and corresponds to the extent of synaptic loss. Microglia also appear to engulf excitatory synaptic components even though the density of excitatory synapses is not affected by cuprizone-induced demyelination, suggesting possible compensatory mechanisms. Following complete remyelination, the density of inhibitory synapses returns to control levels, suggesting recovery of lost synapses, while excitatory synapse density remains unaltered. Our results reveal that microglia are associated with synaptic pathology in MS grey matter, and highlight early synaptic pathology as a therapeutic target in MS to prevent subsequent neurodegeneration.



Graphical Abstract
In homeostasis, microglia are important for the maintenance of synaptic structure and function. During cortical demyelination, inhibitory synapses are selectively lost, while microglia engulf both excitatory and inhibitory synaptic components. Following complete remyelination, inhibitory synapse density returns to control levels, suggesting recovery of lost synapses. Excitatory synapse density is not affected during demyelination/remyelination.

T05-039C

Tubulin beta 4A mutations result in demyelination and neurodegeneration in mouse and human iPSC models

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TUBB4A encodes for tubulin beta 4A, which heterodimerizes with alpha-tubulin and assemble into microtubules (MT). TUBB4A is the predominant beta-tubulin isotype in the human brain and is primarily localized to neurons and oligodendrocytes (OLs). MT are highly dynamic, andessential for neuronal and OL structure, including arborization, polarity, growth cone dynamics,axonal transport and intracellular transport of key proteins. Monoallelic mutations in *TUBB4A* are known to cause a spectrum of neurologic disorders ranging from an early-onset leukoencephalopathy to adult-onset Dystonia type 4. A single recurrent mutation $TUBB4A^{D249N}$ affects majority of patients resulting in Hypomyelinating Atrophy of Basal Ganglia and Cerebellum (H-ABC). Children with $TUBB4A^{D249N}$ exhibit dystonia, gait impairment and cognitive deficits due to loss of neurons and oligodendrocytes in the basal ganglia and

cerebellum. We have developed the first models to study this disease- a *Tubb4a*$^{D249N/D249N}$ knock-in mice and human induced pluripotent stem cell (iPSC) from individuals with *TUBB4A*mutation. Our mouse model recapitulates the progressive motor dysfunction with tremor, dystonia and ataxia seen in H-ABC. *Tubb4a*$^{D249N/D249N}$ mice exhibit myelination deficits due to dramatic decrease in mature oligodendrocytes and their progenitor cells. Further, a significant loss of cerebellar granular neurons and striatal neurons occurs in thesemice. Medium striatal neurons (MSN) derived from*TUBB4A*^{D249N} iPSCs derived undergo apoptosis and a significant decrease in neuronal survival compared to control iPSCs derived MSNs. Thus, we have established both a mouse model and human iPSC-based models to study *TUBB4A*-associated leukodystrophy. Our *in vitro* studies show functional presence of mutant tubulin results in abnormal microtubule dynamics with a cell-autonomous effect of *Tubb4a* mutation in neurons and OLs resulting in reduced survival. Ongoing studies are examining the cellular mechanisms and affected pathways due to defect in this microtubule protein for *TUBB4A*-associated leukodystrophy. In addition, we are testing the use of anti-sense oligonucleotides as a potential therapeutic treatment in both the mouse and human models to ameliorate the disease. Thus, this work is critical to understand the fundamental role of TUBB4A in the neuron and OL health along with application of this research for developing therapeutic avenues.

T05-040C

Post-traumatic brain lesion in a pediatric murine model of injury

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Traumatic brain injury (TBI) is a public health problem since it is the most common cause of disability in patients under 40 years of age. Recently, several studies have demonstrated the development of tertiary lesions occurring up to several years after the TBI, during which a persisting microglial neuroinflammatory phenomenon may be responsible for post-concussive syndrome. We used a model of pediatric TBI on postnatal day (P) seven male mice. We investigate microglial and astrocyte activation, astrogliosis, white matter lesions and behavioral disorders on P45 mice. The study of microglial phenotype, assessed by analysis on gene expression on isolated Cd11b+ cell from the brain, showed that TBI induced a mixed population of microglial cells with pro-inflammatory, anti-inflammatory and immunomodulatory markers particularly three days after the injury; besides seven days after, the TBI microglial activation seemed to be completely resolved. Additionally, immunochemistry labelling revealed an astroglial scar and a neuronal loss in the impact region in association with white matter loss, especially thinning of the corpus callosum and external capsule at P45; electronic microscopy showed also a decrease of myelinated axons in TBI mice. This results were also confirmed in diffusion tensor imaging with an important alteration of the anistrotropic fraction, also functionnal ultrasound imaging with fUS showed brain cerebral connectivity alteration. We recorded ultrasonic vocalizations (USV) at P8 and TBI mice demonstrated a decrease in the number and the duration of USV calls. TBI led to social deficiency at P45 : indeed, TBI mice displayed total indifference to a new mouse during a social interaction test adapted from the three-room test. These results are very promising, and encourage the study of the mechanisms involved in post-concussion syndrome and more particularly the involvement of microglial activation. This study could allow identification of signalling pathways and possibly new therapeutic targets in order to care for post-traumatic disorder patients.

T05-041C

Effects of the GPR17 antagonist montelukast on oligodendrocyte dysfunction and disease outcome in the SOD1G93A mouse model of ALS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the progressive loss of motor neurons (MN) and muscle atrophy, for which no effective therapies are currently available. Recent findings revealed an unexpected early role of oligodendrocyte (OL) dysfunction in the disease progression and death of MNs and suggested that restoring OL function and promoting oligodendrocyte precursor cell (OPC) maturation may open new therapeutic perspectives for ALS (1). An important regulator of OPC differentiation is the P2Y-like GPR17 receptor, which drives the initial steps of this process and it is then downregulated to allow OPC maturation (2). We have recently shown that GPR17 protein levels are pathologically increased in the lumbar spinal cord of SOD1G93A mice at both pre-symptomatic and late symptomatic stages of the disease as compared to littermates expressing the non-mutant form of SOD1 (3). Higher GPR17 levels at early pre-symptomatic stages are coherent with enhanced OPC reactivity aimed at replacing dysfunctional OLs, while prolonged upregulation at terminal stages has been associated with OPC differentiation blockade (2). Importantly, *in vitro* exposure to the non-selective GPR17 antagonist montelukast (MTK) significantly restored the differentiation defects of primary cultured OPCs isolated from SOD1G93A mice, emerging as a good candidate to counteract OL dysfunction in this disease (3). However, an *in vivo* validation of this approach in mouse models of ALS is still missing. Here, we started to evaluate the effects of the *in vivo* administration via oral gavage of two different doses of MTK (10 mg/kg/day and 30 mg/kg/day), once daily from symptoms onset (postnatal day 90) until end stage, in SOD1G93A mice compared to vehicle-treated littermates. Behavioral studies were performed in randomized order by blinded observers three times per week to follow the progression of motor disability. Preliminary results of the analysis of motor coordination by i) rotarod and ii) beam balance tests; motor skills and deficits by i) gait and ii) extension reflex tests; muscle strength by measuring i) pawgrip endurance and ii) grip strength meter, showed signs of improved motor function only after treatment with MTK 30 mg/kg/day dose. Of note, survival probability by the Kaplan Meier analysis slightly but significantly increased in MTK-treated mice. In parallel, immunohistochemical analysis of lumbar spinal cord tissue, collected at postnatal day 110 from SOD1G93A mice treated with MTK or vehicle, are in progress to evaluate the impact of MTK treatment on OL differentiation, myelin integrity and neuroinflammatory parameters, in order to provide insights on the cellular mechanisms underpinning the behavioral improvement observed.

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T05-042C

MCSF prevents Aβ-induced synaptic dysfunction by modifying microglial reactive state

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Accumulation of β-amyloid (Aβ) peptide and synaptic dysfunction are the main hallmarks of Alzheimer´s disease (AD) neuropathology. Loss of synapses occurs early in AD and is considered the best pathological correlate of cognitive decline. However, the role of microglia in AD remains controversial. While microglia mediates early synapse loss in AD models, activation of these cells by immunotherapy or by the cytokine *macrophage colony stimulating factor* (MCSF) results in a more efficient Aβ degradation. Moreover, some studies reported that MCSF treatment reduced Aβ deposits and improved cognitive impairment in AD mouse models.

To study the role of microglial activation by MCSF in Aβ-related synapse pathology, we measured levels of synaptic markers and Aβ by immunofluorescence in primary cultures treated with Aβ oligomers, and carried out immunoprecipitation for extracellular Aβ detection. We found that microglia decreased extracellular Aβ in microglia-neuron co-cultures in presence of extracellular Aβ oligomers, although this was not enough to restore Aβ-induced synapse loss. Synaptic pruning was not observed in our co-culture model. Notably, microglial activation by MCSF was able not only to reduce extracellular Aβ load more consistently but also to prevent synaptic damage. Furthermore, MCSF *in vitro* treatment induced increased Aβ internalization, microglial proliferation and changes in the expression of genes related to microglial activation, inflammatory profile, clearance capacity and complement system.

Additionally, by using two-photon time-lapse imaging in organotypic cortical slices, we detected changes in microglial surveillance and morphology upon MCSF or Aβ treatment. MCSF also reverted Aβ-induced increase in microglial area, measured by Iba1 immunofluorescence in these cultures.

These results strongly suggest that Aβ oligomers are deleterious to synaptic function by interfering with neurons, and that microglial activation by MCSF could constitute an important therapeutic target for the prevention of synapse toxicity.

T05-043D

Cholesterol metabolism, inflammatory response and amyloid-β42 uptake are differentially regulated in isogenic APOE iPSC-astrocytes

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Objectives: The presence of the APOE4 allele is the major risk factor for sporadic Alzheimer's Disease (AD). APOE is mainly expressed by astrocytes in the brain, which play a prominent role in AD, through an exacerbated inflammatory response, lack of synaptic support, cholesterol dysregulation or aberrant Aβ-peptide metabolism. However, the cellular pathogenic contributions of human astrocytic *APOE4* in AD are unclear. Here, we aim to uncover the role of different APOE genotypes (E2, E3, E4 and knockout) in physiological (dys-) functioning of human astrocytes.
Methods: APOE isogenic human induced pluripotent stem cells (iPSCs) are differentiated to functional astrocytes and analysed for glutamate uptake, beta-amyloid uptake, inflammatory signalling as well as cholesterol metabolism. Further, a full proteomic analysis was carried out in astrocytes at baseline and after IL1-β activation.
Results: Astrocyte differentiation potential and proliferation was comparable among lines. Liquid chromatography-mass spectrometry-based proteomic screen combined with gene set enrichment analysis shows genotype-dependent differences in cellular pathways including inflammatory regulation, cholesterol metabolism, energy metabolism, and ECM organization. Moreover, squalene synthase, an enzyme in *de novo* cholesterol synthesis pathway, was reduced in APOE4>E3. On the contrary, cholesterol content was increased in APOE4>E3>E2/KO, where rate limiting enzyme in *de novo* cholesterol biosynthesis HMGCOA Reductase upregulated in APOE4. Furthermore, activated astrocytes showed genotype-dependent increased release of pro-inflammatory cytokines with APOE4>E3>E2>KO, where IL8 and IL6 were released already at baseline in APOE4 cells. Lastly, functional genotype-dependent differences in APOE levels, glutamate uptake, and Aβ uptake were observed.
Conclusions: Cholesterol metabolism shows complex dysregulation in APOE isogenic astrocytes, which can in turn be affected by the APOE allele-dependent aberrant inflammatory response. These dysfunctions go paired with a decrease in homeostatic functioning, leading to a decrease of neuronal support and processing of pathological Aβ42 peptides. Our preliminary data suggest that a gain-of-toxic-function in APOE4 astrocytes may mediate AD pathology relevant processes.

T05-044D

Relative contribution of parenchymal and amyloid plaques associated microglia to Alzheimer Disease progression

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Alzheimer's Disease (AD) is the most common form of dementia. It is characterized by both behavioral deficits (e.g. memory loss) and histological features (e.g. *β*-amyloid deposits and Neurofibrillary Tangles). Neuroinflammation is also recognized as another important hallmark of the disease. The chain of events leading to AD has been the object of intense research for decades. Recently, human genetic studies revealed that microglia, which play key roles in neuroinflammation initiation, express many genes that represent significant risk factors in AD. Those findings support microglia as key contributors to AD pathogenesis. Yet, their contribution to the disease progression is still poorly understood. In particular, whether microglia play beneficial and/or detrimental roles in the disease progression remains heavily debated. One hypothesis to explain this potential duality of effects is that

different subtypes of reactive microglia could play different functional roles.
In AD, microglia clustered around amyloid-*β* plaques show altered phenotypes compared to those distant from the plaques, highlighting the diversity of microglia in this disease. However, the extent to which these two microglia subtypes molecularly and functionally differ remains largely unknown. In this study, we combined laser microdissection and RNA-seq approaches to decipher the roles of plaques-associated microglia and that of parenchymal microglia (i.e. microglia that are not associated with dense plaques). AD is a progressive neurodegenerative disorder, and to take this into account, transcriptional remodeling in the different microglia subtypes has been evaluated at both early, intermediate, and late stages of the disease. By combining WGCNA with GO & pathways analyses, we demonstrated that, as expected from their distinct morphology, plaques-associated microglia exhibit profound transcriptome changes. However, although parenchymal microglia exhibited a typical ramified homeostatic morphology, significant transcriptomic remodeling was also evidenced in this microglia subtype, even in the early stages of the disease when plaques barely form (i.e. 4-months-old APP/PS1 mice).
As a whole, our data support a strong involvement of microglia during AD progression and highlight the differential contribution of parenchymal and plaques-associated microglia to the disease progression. In addition, they confirm that microglia reaction is involved in the early stage of the disease. Identification of microglia subtypes with specific functional roles opens the possibility to target one specific subtype with the aims of either promoting beneficial classes or hampering deleterious ones.

T05-045D

Induced microglia from mononuclear cells as *in vitro* platforms to study inflammatory responses in Alzheimer's patients

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Alzheimer’s disease is the fourth ranked cause of death in society. Microglial activation interacts with amyloid-β (Aβ) fibrils, which is involved in pathogenesis of Alzheimer’s disease (AD). Activation grade of microglia is important; it triggers tissue repair and phagocytosis provides a protective effect in disease pathology but on the other hand overactivation causes inflammation in the central nervous system (CNS). *In vitro* modeling is needed to study microglial functions of Alzheimer's disease pathogenesis and for treatment options, but cells of CNS is hard to use *in vitro*. This study was designed to investigate the roles of microglia in immunological mechanism of Alzheimer's disease in induced microglia like cells derived from monocytes.
Total blood samples from Alzheimer's and MCI patients were used to separate monocytes via density gradient centrifugation and induced microglia was obtained via direct conversion method, using human recombinant IL-34 and GM-CSF. Phenotypical characterization of the induced microglia was done by phase contrast imaging and immunohistochemical analysis. After 15 days of incubation with mentioned cytokines, it was observed that induced cells carry the phenotype of ramified microglia, with wider cell body and neurite outgrowths. Functionality of cells was assessed by changes in the phagocytosis ability and gene expression after stimulation with fibrillar amyloid-β

1-42 peptide and LPS as immune inducers. As a result, it was shown that induced cells show microglia-like characteristics with phagocytosis ability and in their gene expression. In summary, induced microglia cells obtained from AD patients' mononuclear cells were shown to have the characteristics of human ramified microglia, thus presenting a promising option for obtaining an in vitro system for Alzheimer's disease studies in understanding of the pathology, identification of diagnostic and therapeutic strategies.

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T05-046D

Impact of mixed proteinopathies on microglia phenotypes across hippocampal subfields in Alzheimer's Disease and Dementia with Lewy Bodies patients

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The hippocampus, a brain region at the core of the memory process, is particularly vulnerable to neurodegeneration in Alzheimer's Disease (AD) and Dementia with Lewy Bodies (DLB). But if AD and DLB share symptomatic memory decline, the atrophy of the hippocampus does not follow the same pattern with the cornu ammonis (CA) 1 subfield more severely touched in AD patients. To further understand this differential hippocampus deterioration, we have investigated both the distribution of typical AD and DLB proteinopathies and the alterations of microglia phenotypes in a collection of post-mortem human samples AD, DLB, and age-matched controls. Furthermore, because microglia are heterogeneous among brain regions and may respond differentially to proteinopathies, we have analyzed the three subfields of the hippocampus, the dentate gyrus, the CA3, and the CA1.

For this purpose, we have immunostained human post-mortem hippocampal thick fixed sections for amyloid-ß (Aß), phosphorylated forms of tau (Ptau) and a-synuclein (Psyn), and the microglia morphological marker Iba1. We have combined high-resolution confocal microscopy and AI-assisted image analysis tools to characterize the proteinopathy landscape and reveal 3D morphological changes of more than 35,000 individual microglia collected in the 3 hippocampal subregions of 29 individuals including AD and DLB patients and age-matched controls.

We found a more complex pathological environment than expected with a general but variable presence of mixed protein inclusions, i.e Aß plaques, neurofibrillary tangles, and Lewy bodies, across subfields and in both conditions. Our volumetric analysis of proteinopathy burdens shows subfield- and condition-dependent patterns with AD CA1 being characterized by a higher level of PTau. Additionally, we have analyzed the changes of 16 morphological features for each microglia and identified trends towards a less complex morphology, with fewer and shorter branches, loss of polarity, and ameboid-like shape in both diseases but with statistical significance only in AD.

Some of these morphological alterations were found more pronounced in certain hippocampal subfields. We have also found an association between Aß, Ptau and morphological features with correlation analysis.
We show here that microglia changes are tightly regulated by their immediate proteinopathy environment. However, certain alterations are specifically exacerbated in some hippocampal subfields and neurodegenerative diseases. This study highlights the complexity of AD and DLB pathological changes and the regional and disease-specificity of human microglia phenotypes.

T05-047D

Glial dysregulation and SVZ neurogenesis disruption following brain chemical lesion

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Long-term adverse side effects of chemotherapy, also known as "Chemo-Brain", have been only recently anticipated and several mechanisms are proposed to be involved in changes regarding brain structure and function following systematic use of chemotherapeutic agents. These include reduction of Neural Stem Cell (NSC) proliferation rates in adult brain neurogenic zones, white matter degeneration and inflammation. This phenomenon is particularly pronounced in cancers, such as glioblastoma, which are inherently resistant to chemotherapy requiring high doses of chemotherapy to eliminate them, resulting in its high concentration in the cerebrospinal fluid.
Here we aimed to study the effect of brain chemical lesion induced by multiple stereotaxic intraventricular injections of the chemotherapeutic mito-toxic agent arabinoside-C on adjacent neurogenic and non-neurogenic brain areas fine cytoarchitecture and their relation to the functional outputs of 'chemobrain'. Characterization of the spatio-temporal distribution of neuronal and glial cells at several multiple time points following the chemical lesions (4, 15 days and 6 weeks) revealed that Ara-C administration leads to ependymal cell layer disruption, extensive astro-micro gliosis, glial dysregulation and myelin impairment. Moreover it triggers doublecortin+ (DCX+) neuroblasts' ectopic presence in the adjacent to the SVZ non-neurogenic subcortical parenchyma, the majority of which cluster inside myelinated white matter tracts. Our studies are on-going to characterize the lineage trajectories of ectopic neuroblasts and evaluate myelin microstructure and migrating neuroblasts by using transmission electron microscopy (TEM). We anticipate that the analysis of the brain cytoarchitecture, as well as the lineage, molecular phenotype and proliferation properties of SVZ and striatal cell populations will provide us with answers linking brain morphology to function following chemotherapy.

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T05-048D

Reactomics of the multimodal regulation of astrocytic GLT1 expression by β-amyloids

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We consider the molecular underpinnings for the expression of astrocytic glutamate transporters (GLT1) upon modulation of extracellular amyloid-beta (eAβ) oligomers as a mapping of Alzheimer's disease (AD) progression. With this aim, we characterize surface vs. cell-wide GLT1 expression in astrocyte cultures for different applications of eAβ in duration and concentration. The quantification of such expression along the eAbxTime axis reveals different modes of trafficking of GLT1 between the surface vs. intracellular compartments. To gain insights into the molecular underpinnings of such variegated trafficking, we combine *in silico* approaches with single-cell genomics analysis. Fitting a Markov model of GLT1 trafficking with our data identifies multiple causal pathways for astrocytic transporter dynamics with our in vitro model of AD progression. We thus, resort to signal-enriched genomics analysis to identify putative overlaps between the model's predicted pathways and useful molecular signals to resolve the reactome accounting for the hierarchical organization of GLT1 trafficking modes. The analysis identifies astrocytic signatures of AD evolution, pinpointing new venues for diagnosis of preclinical AD stages by neuron-glial markers.

T05-049D

Mild Microglial Responses in the Cortex and Perivascular Macrophage Infiltration in Subcortical White Matter in Dogs with Age-Related Dementia Modelling Early Alzheimer's Disease

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Background: Microglia contribute to Alzheimer's disease (AD) pathogenesis by responding to and clearing

Amyloid-β (Aβ) and driving neuroinflammation, but the mechanisms how microglia interact with A*β* are far from understood. Studies of domestic dogs with age related dementia (canine cognitive dysfunction (CCD)), which also develops cerebral amyloid angiopathy, can provide novel information about microglial response in early-stage AD.
Objective: The aim was to investigate the microglial response to the cognitive status and Aβ accumulation in the cortical grey and subcortical white matter in dogs with CCD compared with age-matched cognitive normal dogs.
Methods: Frontal cortex sections from old dogs with CCD and age-matched controls were studied. Cases were defined by a CCD rating scale (CCDR). Sections were stained immunohistochemically for Aβ (6E10) and the microglial-macrophage ionized calcium binding adaptor molecule 1 (Iba1). Results were correlated to CCDR score and previously collected data on prefrontal cortex tissue levels of Aβ42 and N-terminal pyroglutamate modified Aβ(N3pE)-42.
Results: Microglial numbers were higher in the Aβ plaque loaded deep cortical layers in CCD versus control dogs, while coverage by microglial processes remained unaffected. There was no microglial aggregation around the Aβ plaques. However, a correlation was found between the % area covered with Iba1 immunoreactivity and the levels of insoluble Aβ42 and Aβ(N3pE)-42. The % Iba1 area was higher in white versus grey matter. Furthermore, perivascular macrophage infiltrates were particularly abundant in the white matter in CDD dogs.
Conclusion: The results from this study of the microglial-macrophage response in dogs with CCD might contribute to knowledge on early-stage AD as a relative mild microglial response in the Aβ plaque loaded deep cortical layers and perivascular macrophage infiltrates in the subcortical white matter were observed.

T05-050D

Deficient metabolic plasticity of glial cells in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive and selective loss of motor neurons in the motor cortex, the brain stem, and the spinal cord. This results in muscle denervation, leading to progressive weakness, atrophy, and death, typically within few years after diagnosis. Despite our increasing understanding of the cellular and molecular mechanisms contributing to ALS, no effective treatment is currently available. One of the hallmarks of ALS is the profound dysregulation of energy metabolism that is commonly observed both in patients and in animal models of the disease. Hence, ALS patients show considerable weight loss, which is frequently attributed to insufficient food intake, but also to a general hypermetabolism. Furthermore, neurodegeneration in ALS has been shown to involve “non-cell autonomous” mechanisms, meaning that altered activity of other cells in the nervous parenchyma has a dramatic impact on neuron survival. Considering the key role played by astrocytes in the control of energy metabolism in the nervous system, it is surprising that the study of glial cell metabolism has received little attention in the context of ALS. We herein propose that a major deficiency in the metabolic plasticity of astrocytes in ALS contributes to the loss of physiological support of nearby neurons, exacerbating their vulnerability to stress.
While some metabolic pathways are already known to be altered in motor neurons in the context of ALS, more recent studies have been focusing on the implication of the key modulator of cell metabolism: AMP-activated

protein kinase (AMPK). Alterations in the expression and/or activity of this enzyme have already been reported in motor neurons, both in ALS patients and animal models of the disease. Considering that neurons mainly rely on astrocytes for metabolic support, understanding AMPK regulation in glial cells should also be considered.
Since little is known about AMPK in glial cells, we first characterized its expression in primary cultures of astrocytes derived from wild-type rats or transgenic rats carrying an ALS-associated mutated hSOD1 (hSOD1G93A). Our data showed that mRNA levels encoding for the alpha1 and alpha2 catalytic subunits of AMPK are altered in cultures from the transgenic animals. Then, as AMPK is influenced by the energy status of the cell, we characterized its activity in astrocytes subjected to metabolic stress conditions (i.e. glucose deprivation). Even though further experiments are being conducted to investigate the underlying molecular mechanisms, our results suggest that astrocytes from ALS rodents are poorly equipped to face metabolic stresses, compared to their wild-type counterparts.

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T05-051D

Cell-specific cholesterol synthesis ablation affects cerebral β-amyloidosis

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Cholesterol homeostasis within the CNS is maintained by de novo synthesis of most if not by all cells of the brain. Alterations of cholesterol metabolism are linked to the pathogenesis of Alzheimer´s disease (AD). One of the hallmarks of AD are extracellular amyloid plaques that contain amyloid β-peptides. Amyloid β-peptides can bind cholesterol and amyloid precursor protein (APP) predominantly segregates to cholesterol-rich membrane lipid rafts. To uncover the connection between β-amyloid pathology and cell-type specific cholesterol homeostasis, we combined the 5xFAD mouse model of AD with mutants lacking cholesterol synthesis in neurons, astrocytes, and microglia. 5xFAD mice harboring AD-linked mutations in the human APP and presenilin-1 (PSEN1), causing an early onset of β-amyloid pathology. Although altered neuronal cholesterol levels modify APP processing in vitro, ablation of neuronal cholesterol synthesis surprisingly did not affect amyloidosis in compound mutants. In contrast, glial cholesterol synthesis affected amyloid pathology. Loss of cholesterol synthesis in astrocytes reduced the amyloid burden in 5xFAD mice. Surprisingly, amyloid-β plaque load was extensively increased in microglial cholesterol mutants. Microglia are involved in compaction, recognition, uptake, and phagocytosis of β-amyloid. Bulk sequencing data of microglia synthesis mutants revealed alteration of the microglial activation profile and DAM signature genes. Taken together, our data suggests that modulating cellular sterol synthesis interferes with β-amyloid pathology and could serve as a promising target for therapy approaches.

T05-052D

The Sonic Hedgehog agonist SAG attenuates mitochondrial dysfunction and decreases the neurotoxicity induced by frataxin-deficient astrocytes

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Friedreich's ataxia (FRDA) is predominantly a neurodegenerative disease caused by the deficiency of a protein called frataxin (FXN). Although the main pathological alterations are observed in neurons, it is becoming clear that other non-neuronal cells such as astrocytes may be actively involved in the neurodegenerative process associated with the disease.

Depending on the stimuli they respond to, astrocytes acquire different activation states in a process called astrogliosis. Neuroinflammatory stimuli induce the formation of A1 reactive astrocytes, which upregulate proinflammatory genes, being harmful for neurons. A1 astrocytes have been detected in post-mortem tissue of patients with different neurodegenerative disorders, being hypothesized that they might have deleterious effects on neurons, exacerbating the neurodegenerative process. Recent studies have demonstrated positive effects of Sonic Hedgehog (SHH) agonists in astrocyte viability and proliferation, astrocyte-mediated neuroprotection, and also positive effects in mitochondrial activity and dynamics. As mitochondrial changes are important components in the etiology of neurodegenerative disorders, the influence of SHH agonists in mitochondrial physiology could be of therapeutic relevance. In this work, we have thoroughly characterized astrocyte reactivity phenotype and mitochondrial status of FXN-deficient human astrocytes, evaluating as well the effect of SHH agonists on astrocyte reactivity, viability, and function.

We used an *in vitro* model based on a short hairpin RNA packaged in a lentiviral vector, which allowed us to decrease FXN levels in human cortical astrocytes, to similar levels as those observed in FRDA patients, and found that FXN-deficient cells had less cell viability and higher expression of several A1 reactive astrocyte markers, than control cells. Both phenomena were prevented by a chronic treatment with the smoothened agonist (SAG), a SHH signaling agonist. Moreover, FXN-deficient astrocytes showed defects in mitochondrial function and dynamics, which were partially rescued by SAG. Regarding the possible neuroprotective effects of SHH agonists, previous results showed that FXN-deficient astrocytes are able to induce neurodegeneration, and we have observed that the chronic treatment with SAG attenuated the neurotoxicity triggered by the treatment of mouse cortical neurons with conditioned medium of FXN-deficient astrocytes.Overall, our results suggest that the treatment of FXN-deficient astrocytes with a SHH agonist like SAG, could be used as a possible target to reduce FRDA-associated neurodegeneration.

T05-053D

Parkinson's Disease–Associated LRRK2 Interferes with Astrocyte-Mediated Alpha-Synuclein Clearance

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The elimination of unwanted and potentially harmful material is crucial for nervous system development and function. Neuronal and glial proteinaceous fibrils mainly composed by α-synuclein (α-syn) are the pathological hallmarks of Parkinson's disease. Of note, toxic α-syn can be released by cells and spread through the brain contributing to disease progression. Compelling evidence suggest that astrocytes engulf α-syn aggregates and partially degrade them via the lysosomal pathway.[1-2] However, the molecular mechanism behind astrocyte-mediated α-syn clearance remains unclear. In this context, we provided evidence that LRRK2, a kinase involved in genetic forms of PD, intervenes in this pathway. We compare the capacity of wild-type astrocytes and astrocytes carrying the PD-linked G2019S mutation in Lrrk2 to ingest and degrade fibrillary α-syn. Our results show that astrocytes carrying the G2019S mutation in Lrrk2 exhibit a decreased capacity to internalize and degrade fibrillar α-syn through the endo-lysosomal pathway. In addition, we demonstrate that the reduction of α-syn internalization in the Lrrk2 G2019S astrocytes is linked to annexin A2 (AnxA2) loss of function. Noteworthy, the pathogenic phenotype is fully reverted by the addition of a selective LRRK2 kinase inhibitor. Together, our findings reveal that astrocytic G2019S LRRK2 interferes with the clearance of extracellular α-syn aggregates through an AnxA2-dependent mechanism. Moreover, the pathogenic mechanism is linked to the elevated kinase activity that distinguishes the LRRK2 mutated forms.[3]

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T05-054D

The glutamate transporter recycling is delayed by pathogenic LRRK2

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Excitatory amino acid transporter 2 (EAAT2 in humans, GLT-1 in rodents) accounts for the 90% of brain glutamate clearance and it is mainly expressed on the astrocytes perisynaptic processes. Dysregulation of EAAT2 has been associated to impaired glutamatergic neurotransmission, excitotoxicity, inflammation as well as neurodegeneration. As many receptors and transporters, EAAT2 is constitutively internalized and partially recycled back to the plasma membrane or degraded. With this study we revealed that EAAT2 recycling is perturbed by the pathogenic G2019S Leucine rich repeat kinase 2 (LRRK2), a mutated kinase involved in genetic Parkinson's disease (PD). By combining molecular and imaging techniques, we showed that EAAT2 level is strongly decreased in G2019S LRRK2-linked PD brains and matches with elevated gliosis. Moreover, voltage-clamp recording in *Xenopus laevis* oocytes co-injected with EAAT2 and LRRK2 *wild-type* or G2019S mRNA revealed that LRRK2 mutation impinges on EAAT2-associated current interfering with its localization at the plasma membrane. We also demonstrated that a similar phenotype occurs in young Lrrk2 G2019S mice in the absence of dopaminergic neurodegeneration and correlates with a reduction of glutamate reuptake by gliosomes (purified astrocytic terminals). Using imaging techniques, we showed that Glt-1 accumulated in the intracellular space in Rab4-positive fast-recycling vesicles and its recycling kinetic was strongly delayed in G2019S Lrrk2 primary striatal astrocytes. Of note, the pharmacological inhibition of Lrrk2 kinase reverted the pathological phenotype, pointing to Lrrk2 as a good target to slow down early glutamatergic phenotypes in Lrrk2-linked PD. Overall, our results unravel a novel pathway deregulated in PD and open a new therapeutic window for a disease that, at present, has no cure.

T05-055D

Effect of *Vitis Vinifera* Grape Seed Extract on brain and spinal cord demyelination and oxidative stress parameters in an experimental autoimmune encephalomyelitis mouse model

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Background and aims:
Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) that causes different debilitating symptoms including neuropathic pain found in 47% of patients[i]. Grape Seed Extract (GSE) is an extract prepared from the seeds of red grapes (Vitis Vinifera), which have shown antioxidant and anti-inflammatory effects in different disease models thanks to its richness in phenolic compounds[ii]. Therefore, our aim is to characterize effect of GSE in an experimental autoimmune encephalomyelitis (EAE) mouse model of MS with neuropathic pain symptoms.
Methods: Effects of early curative treatment with GSE (500 mg/kg/day) from 10 to 30 days post EAE induction has been evaluated on clinical scores, motor performances as well as paw sensitivity to mechanical and thermal stimuli. We then, characterized the GSE cellular effect on brain and spinal cord samples focusing on demyelination and oxidative stress pathways using both biochemical and cellular biology techniques. We notably evaluated lipids peroxidation, oxidative damage of protein and antioxidant enzymes activities as well as target proteins expression.
Results:
We showed in treated EAE mice that GSE: significantly ameliorates motor clinical symptoms, totally reverses mechanical allodynia and partially improves cold allodynia and thermal heat hypersensitivity. Until know, these beneficial effects have been linked to: 1) a reduction in the level of carbonylated proteins; 2) a correction of the decreased catalase activity; 3) the reduction of demyelination and the associated astrogliosis observed in untreated EAE brain and spinal cord.
Conclusion:
We then showed for the first time that GSE cannot only improves the EAE clinical scores but also ameliorates nociceptive symptoms. We are currently describing the cellular basis of this therapeutic effect, as such, a proteomic analysis is planned in order to better identify altered signaling pathways present in our model and potentially corrected by GSE.
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T05-056D

Loss of TDP-43 in astrocytes leads to motor deficits by triggering A1-like reactive phenotype and tri-glial dysfunction

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Patients with amyotrophic lateral sclerosis (ALS) can have abnormal TDP-43 aggregates in the nucleus and/or cytosol of their surviving neurons and glia. Although accumulating evidence indicates that astroglial dysfunction contributes to motor neuron degeneration in ALS, the normal function of TDP-43 in astrocytes are largely unknown, and the role of astroglial TDP-43 loss to ALS pathobiology remains to be clarified. Herein, we show that TDP-43-deleted astrocytes exhibit a cell autonomous increase in GFAP immunoreactivity without affecting astrocyte or

microglia proliferation. At the transcriptomic level, TDP-43-deleted astrocytes resemble A1-reactive astrocytes and induce microglia to increase C1q expression. These astrocytic changes do not cause loss of motor neurons in the spinal cord or denervation at the neuromuscular junction. In contrast, there is a selective reduction of mature oligodendrocytes, but not oligodendrocyte precursor cells, suggesting tri-glial dysfunction mediated by TDP-43 loss in astrocytes. Moreover, mice with astroglial TDP-43 deletion develop motor, but not sensory, deficits. Taken together, our results demonstrate that TDP-43 is required to maintain the protective functions of astrocytes relevant to the development of motor deficits in mice.

Acknowledgement

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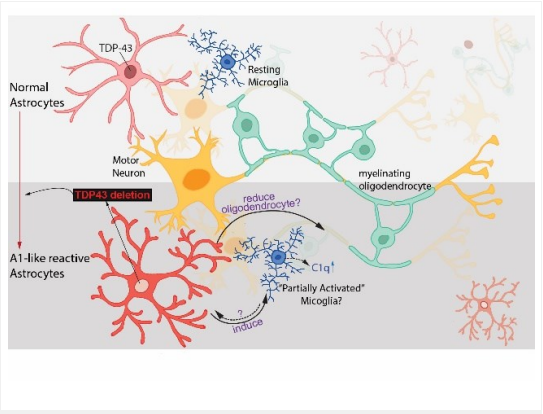

Proposed working model of tri-glial dysfunction triggered by astroglial TDP-43 deletion
Astrocytes without TDP-43 show enhanced GFAP immunoreactivity and acquire an A1-reactive signature, which induces microglia to increase C1 complement system expression and leads to a reduction of mature oligodendrocytes.

T05-057E

Role of SHIP1 in modulating microglial function

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Microglia are the innate immune cells of the central nervous system (CNS). They not only play crucial roles in brain development, repair and plasticity, but are also critically involved in neurodegenerative processes. Genome wide associations studies (GWAS) reveal that hundreds of genetic variants associated with neurodegeneration are highly expressed in microglia. Nevertheless, the functions of most of these disease risk genes have been so far poorly studied. Therefore, we selected a set of highly expressed microglial genes, whose SNPs (single nucleotide polymorphisms) have been associated with cognitive impairment, to investigate their role as modulators of microglial function. We used a loss-of-function approach, using CRISPR/Cas9 genome editing in BV2 cell line, to assess the implication of those genes in regulating basic properties such as motility, phagocytosis and degradation.

Here, we focus on the role of SHIP1, encoded by Inpp5d, responsible for the dephosphorylation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to PIP2. We have generated three single clones knock-out for Inpp5d. First, we characterized the uptake and degradative capacity in relation to different cargoes (such as synaptosomes and amyloid beta), showing alterations as compared to their scrambled controls. Furthermore, we investigated possible differences in endo/lysosomes acidification at baseline and in nutrient-limiting conditions. In addition, we identified an impairment in motility by means of wound healing assay.
In conclusion, this study shows that SHIP1 plays an important role in modulating microglial properties.

T05-058E

Exploring the link between myelin biology and Alzheimer's disease.

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Although amyloid ß- peptide (Aß) accumulation remains the prevalent hypothesis for the initiation of Alzheimer's disease (AD), it does not explain the whole picture, and the pathogenesis of AD is still far from being fully understood. Almost all animal studies on AD rely on transgenic rodents targeting Aß biosynthesis pathway, inducing a strong bias that may impede the progression of our knowledge on the sporadic form of AD which represents 95% of cases [1]. Because of numerous observations suggest links between myelin, cognition and AD, we propose to investigate if and how myelin biology (oligodendrogenesis, myelin remodeling) contribute to AD-induced neurodegeneration [2,3].
In a first step we examined myelin content in a non-transgenic AD mouse model obtained by streptozotocin (STZ) intracerebroventricular injections [4]. Compared to vehicle injected mice we observed reduced myelin content in the corpus callosum of STZ mice before the decrease in synaptophysin and the increase in Aß and Phosphorylated Tau in the cortex, suggesting early myelin alteration in this AD model. Long term effect of an episode of demyelination in mice is known to induce altered pattern of myelin [5]. To examine whether this model is more susceptible to develop AD hallmarks, we challenged oligodendrogenesis either by inducing a transient demyelination using cuprizone intoxication. Mice were then submitted to cognitive evaluation in a variety of behavioral tests (open maze, Y maze, novel object recognition and Morris water maze) before being sacrificed for biochemical analyses of brain tissue. Interstingly, we observed that from 2 to 3,5 months after the end of cuprizone treatment, at a time point when remyelination is complete, cuprizone fed mice exhibited similar cognitive defaults as STZ mice, with reduced spontaneous alternation in the Y maze, reduced spatial memory and lack of flexibility in the watermaze. Biochemical analysis of cortex and hippocampus tissues for quantification of AD and myelin markers are ongoing.
Our preliminary results support the hypothesis that myelin dysfunction may underlie or contribute to AD development.

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T05-059E

Accumulation of neuronal debris in astrocytes - a possible mechanism for spreading of tau pathology

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The key neuropathological hallmarks of Alzheimer's disease (AD) are extracellular plaques, mainly consisting of aggregated amyloid β (Aβ) and intracellular neurofibrillary tangles, composed of aggregated tau. Decades of research have focused on neuronal abnormalities in AD pathology, but recently more attention has been given to glial cells, including astrocytes. The overall aim of this study is to examine the role of astrocytes in the propagation of tau pathology. Since astrocytes do not express tau, the astrocytic tau inclusions, frequently found in the human AD brain, is considered to be of neuronal origin. However, the mechanism behind the appearance of astrocytic tau deposits and their relevance for disease progression remains unknown. We have previously shown that astrocytes, in addition to aggregated proteins, effectively engulf dead cells. In this project, we are investigating if human iPSC-derived astrocytes promote cell-to-cell spreading of toxic tau aggregates following ingestion of dead neurons with tau pathology. Hence, the first goal was to induce robust tau pathology in cultured human iPSC-derived neurons. Then we induce apoptosis and present them to human astrocytes. For this purpose, neurons were exposed to different types of synthetic tau seeds, including tau-oligomers and sonicated tau-fibrils. Already after 48h, there were significant morphological and synaptic changes in the neurons treated with tau-oligomers compared to control, and at day 7 there was a widespread neuronal cell death. In contrast, neurons treated with tau-fibrils showed no significant difference compared to control. Moreover, immunocytochemistry using various tau antibodies suggested a significant increase in phosphorylated tau following oligomer exposure. Next, we investigated the mechanisms by which human astrocytes handle neuronal corpses. Neuronal apoptosis was induced by exposure to a short UV-burst (480 mJ) followed by the addition of astrocytes to the culture. After 7 days, we measured the average number of engulfed dead neurons inside each astrocyte. Although, the astrocytes ingested several cell corpses, there was minimal degradation of these engulfed dead neurons over time. Taken together, our data suggest that tau-oligomers are more neurotoxic than tau-fibrils and that neurons treated with tau-oligomers have increased levels of phosphorylated tau, which is believed to be the first step in the induction of tau pathology. Finally, our data suggests that astrocytes are able to engulf neuron corpses, which may lead to the spreading of toxic prion-like tau species.

T05-060E

Redox imaging in 5xFAD mice reveals close associations between microglia, highly oxidized dystrophic neurites and amyloid plaques

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Intense oxidative stress occurs at Alzheimer's Disease (AD) plaques contributing to synaptic dysfunction and neuronal death yet the triggers for generating reactive oxygen species (ROS) remain elusive. Microglia are thought to contribute to AD pathology and to modify ROS generation by acquiring neuroprotective or neurotoxic phenotypes. Here we report new imaging strategies using genetically encoded roGFP redox sensors to simultaneously image microglia and neuronal redox states in live cells *in vivo* and in paraformaldehyde fixed brain tissue with additional immunocytochemistry. By employing these tools, we found that microglia preferentially surround and engulf dystrophic neurites (DNs) with the greatest degree of cytoplasmic oxidation around amyloid plaques in 5xFAD mice. High levels of cytoplasmic oxidation in DNs correlated with elevated LAMP1 and ubiquitin thereby linking dysfunctional lysosomes to oxidative stress in DNs. Long-term *in vivo* 2-photon imaging revealed highly dynamic interactions between microglia and DNs. When microglia were depleted from 5xFAD mice by administering PLX3397, oxidative stress surprisingly was exacerbated which correlated with even greater elevations of LAMP1. Interestingly, we also confirmed the previously reported neuroprotective effect of microglia depletion in 5xFAD mice and found reduced levels of neuronal loss after treatment. Our data highlights two seemingly paradoxical functions of microglia in 5xFAD mice: While microglia ameliorate redox stress in DNs they also promote the loss of neurons. Our novel approaches to study microglia and neuronal redox levels simultaneously allow to further dissect the underlying redox mechanisms and identify key pathways of neurotoxic and neuroprotective microglial functions in AD.

T05-061E

Development of cutting-edge editing technologies to treat Alexander disease

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Alexander disease (AxD) is an autosomal dominant neurodegenerative disorder caused by heterozygous missense mutations in the gene encoding the glial fibrillary acidic protein (GFAP), the major intermediate filament protein in astrocytes. In AxD patients, GFAP mutations are mainly clustered in two hotspots affecting arginines at positions 79 and 239 of the structural helical coiled-coil rod domains, which play a crucial role in filament assembly. Accumulation of GFAP aggregates in Rosenthal fibers leads to impairment of proteasomal activity and hyperactivation of the stress response, thus compromising astrocyte functions and altering the homeostasis of the central nervous system (CNS) in AxD patients and murine models. Currently, this is an orphan disease that lacks a cure.

Our long-term goal is to develop novel gene editing strategies targeting GFAP mutational hotspots to recover pathological phenotypes in astrocytes. To this end, we are exploiting CRISPR-associated Cas9 nucleases, which enable precise genome editing by inducing DNA double strand breaks (DSBs) at selected genomic loci. DSB can be then repaired by the error-prone non-homologous end-joining (NHEJ) pathway leading to knock-down of target genes.

Here, we engineered murine cell lines to express mutated and wild-type (WT) *Gfap* coding sequences fused to reporter genes for a fast screening of single guide RNAs (sgRNAs) recognized by different Cas9 nucleases. We identified CRISPR/Cas9 systems efficiently inducing the allele-specific knock-out of mutated R76H or R236H *Gfap* sequences, without off target activity on the WT *Gfap* gene. As alternative editing strategy, we are currently testing adenine base editors (ABEs) to correct R-to-H mutations at the *Gfap* mutation hotspots, thus avoiding the potential activation of DSB-induced DNA damage response and apoptosis in editing cells.

These preliminary *in vitro* data will pave the way to translational studies aimed at demonstrating the efficiency, efficacy and safety of the intracerebral injection of AAV vectors or nanoparticles carrying the selected editing systems in AxD murine models. These novel editing platforms for *in vivo* targeting of CNS astrocytes could be applied prospectively for the development of novel, single-dose therapeutic treatments for AxD and other neurodegenerative disorders characterized by primary astrocyte degeneration or dysfunctional/maladaptive astrogliosis.

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T05-062E

The nigrostriatal pathway of aged system x_c^- - deficient mice is not protected against MPTP-induced toxicity

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Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder. Despite significant progress made over the past decade in understanding the molecular causes of nigral dopamine (DA)-ergic degeneration in PD, there are currently no neuroprotective interventions that can halt disease progression in patients. Promising findings in pre-clinical animal studies have consistently failed to translate into efficient interventions in clinical trials. Various explanations have been proposed for the failed translation of promising neuroprotective targets, including reduced external validity of the animal models used. In addition, pre-clinical studies have been largely performed in young animals, and are very biased on using male animals, whereas clinical trials enroll a heterogeneous population of patients of both genders, during their late life.

The glial cystine/glutamate antiporter system x_c^-, with xCT as specific subunit, exchanges extracellular cystine for intracellular glutamate and has been shown to be the main source of extracellular glutamate in the striatum of mice. System x_c^- is enhanced in stress conditions; the resulting increase in extracellular glutamate levels can induce excitotoxicity and drive neuroinflammation. Accordingly, genetic deletion of xCT has been shown to be protective in

mouse models for neurological diseases, including PD (Massie *et al*. (2011) FASEB J 25: 1359-69). This protection was, however, not confirmed in the chronic, progressive MPTP model applied to young mice (Bentea *et al*. (2015) Neuroscience Letters 593, 1-6).

In the current study we adapted this model for use in aged mice and investigated the susceptibility of aged $xCT^{+/+}$ and $xCT^{-/-}$ mice to MPTP-induced nigrostriatal degeneration. Seventeen-month-old mice were injected with saline or MPTP daily for a period of three weeks. Each week the dose of MPTP increased from 10, 20 to 24 mg/kg/day for 5 days. Next, motor function was evaluated using the DigiGait system that analyzes the gait of the mice. *Post-mortem*, nigrostriatal degeneration was evaluated by immunohistochemistry for tyrosine hydroxylase (TH), a marker for DA-ergic neurons. xCT expression was analyzed using semi-quantitative Western blotting.

The first results of this study indicate that xCT deletion in aged mice is not protective in the chronic, progressive MPTP model, contrary to what we previously observed in other PD models. Three weeks of MPTP injections induced a 40% loss in striatal TH expression and approximately 20% of nigral DA-ergic cell loss, independent of genotype. Further analyses are required to elucidate the discrepancy between different models.

T05-063E

Differential eIF2B-mediated translational control between neuronal and glial cells during stress and disease

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Initiation of protein synthesis is regulated by eIF2B, a guanine-nucleotide exchange factor (GEF) required to recycle eIF2. Cellular stresses such as ER stress and oxidative stress activate the Integrated Stress Response (ISR): eIF2α is phosphorylated and blocks eIF2B activity, inhibiting protein synthesis and inducing ATF4-CHOP-regulated signature cascade to promote cell recovery (GADD34) or apoptosis. Abnormalities in the ISR have been reported in neurodegenerative diseases such as Alzheimer's, Parkinson's and disorders of myelinating glia such as Pelizaeus-Merzbacher disease. Mutations in subunits of eIF2B are associated with leukoencephalopathy with vanishing white matter (VWM), a progressive neurodegenerative disease where neurological deterioration is exacerbated upon episodes of acute stress (*e.g.* head trauma). VWM primarily manifests as a leukodystrophy characterized by immature astrocytes with a strongly activated ISR and an increased number of oligodendrocyte progenitor cells (OPCs), whilst neurons remain largely unaffected.

We previously showed that eIF2B localizes in cytoplasmic foci complexes termed eIF2B bodies. These eIF2B bodies vary in size and composition of eIF2B subunits (α-ε). Upon induction of the ISR, eIF2Bδ increases its distribution to small-sized bodies in astrocytes. Our current data highlights that neurons have distinct eIF2B body patterns and higher co-localisation of eIF2Bδ to small bodies in neurons, in the absence of stress, in comparison to glial cells. We also observe that neuronal cells preconditioned with chronic ER stress showed protective features upon a short-term acute stress episode, with milder or no beneficial effect on glial cells. Future work aims to track eIF2Bδ under these stress environments and further investigate its functional role on the ISR.

The molecular mechanisms of VWM are poorly understood, with specific mutations that cause severe forms of the disease having no effect on the currently characterised functions of eIF2B. Herein, we show that eIF2B localisation is disrupted by specific VWM mutations in glial cell lines.

Taken together, our study has shown that eIF2B bodies are dynamically modulated in a cell type-dependent manner and gives preliminary data on a potential novel mode of action of eIF2Bδ during the ISR which could be relevant to the VWM-glial sensitivity.

T05-064E

Effects of alpha-synuclein on astrocytic-neuronal crosstalk

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Parkinson's disease has been primarily regarded as a neuronal pathology but recent evidence suggests that the disrupted interaction between neurons and glial cells, in particular astrocytes, might contribute to the disease progression. Importantly, astrocytes play a pivotal role in ensuring proper neuronal functions by means of their metabolism, which encompasses processes such as neurovascular and energetic coupling. Among the genes associated with familial PD, *SNCA* is one of the most widely studied in the field. This is explained by the fact that *SNCA* codes for α-synuclein – the main component of Lewy bodies, which are a hallmark of PD. Although α-synuclein is mostly expressed in neurons, some studies suggest that even low levels of α-synuclein in glial cells might contribute to disease pathology. Furthermore, it has been shown that astrocytes can engulf α-synuclein secreted by neurons, further supporting the notion that astrocytes might be important players in the etiology of PD. Interestingly, in iPSC-derived neurons, α-synuclein has been implicated in mitochondrial dysfunction as well as ER-mitochondria contact site maintenance. By contrast, its impact on mitochondrial homeostasis in astrocytes has not been studied in detail.
In the light of these findings, we aim to study the neuronal-astrocytic cooperation in the context of PD using patient-derived models harboring SNCA mutations. Thereby, we will especially focus on metabolic alterations, which could exacerbate neuronal pathologies. Applying established protocols, we differentiated iPSCs into non-activated patient and control astrocytes as well as dopaminergic neurons. Performing high-content confocal imaging using astrocytic and neuronal markers, we assessed the composition of these cultures. Next, we are planning to study metabolism as well as mitochondrial function in patient and control astrocytes and neurons with the aim to unravel the impact of mutant α-synuclein on the crosstalk of these cells.

T05-065E

Identifying and controlling regulators of reactive astrocyte state change.

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In neurodegenerative disease astrocytes change states to become reactive and can contribute to progressive neurodegeneration. Modulating astrocyte cell state by blocking the formation of these pathological reactive astrocytes thereby represents an attractive therapeutic goal. We have leveraged a novel cellular platform to further understand the epigenetic and transcriptional regulation of reactive astrocyte state change and identify therapeutic

targets to inhibit this state change. Here we show that during a reactive state change, astrocytes undergo significant chromatin reorganization that drives expression of genes responsible for canonical reactive astrocyte functions. We also identify small molecules that inhibit key regulators required for reactive astrocyte state change and that lead to improved axonal health and increased repair *in vivo* following central nervous system injury. Collectively, these results provide greater mechanistic understanding of reactive astrocyte state change and identify potential reactive astrocyte targeted therapeutics for neurodegenerative disease.

T05-066E

Analysis of autophagy and cytotoxicity in apolipoprotein E ε4/ε4 iPSC-derived mature human astrocytes in response to α-synuclein

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Astrocytes remove and degrade extracellular α-synuclein (αS) before it can aggregate and cause cell death. It has previously been shown the apolipoprotein E (apoE) ε4 allele increases the risk of synuclein pathology in neurodegenerative diseases such as dementia with Lewy bodies. Also, apoE is an integral part of the autophagosome, with evidence indicating apoE ε4 expression results in inhibition of autophagic flux which results in protein aggregation. Initial studies in control human cortical astrocytes demonstrated that apoE secretion was decreased in response to physiological levels of αS, with subsequent increases in lysosome marker LC3, indicating autophagosome formation, and GFAP, a marker for astrogliosis. Likewise, cytotoxicity was not different than control treatment, indicating cells did not transition to cell death. To determine if αS degradation by astrocytes was differentially affected by the apoE ε4 allele, mature astrocytes derived from induced pluripotent stem cells (iPSCs) were challenged with increasing concentrations of 100 nM, 250 nM and 500 nM αS for 24 hours. Cells were first transfected to express red fluorescent protein (RFP) fused to Lamp1 and treated in conjunction with HyLite fluor 488 labeled αS to discern if αS colocalized with lysosomes. Colocalization occurred at all treatment conditions, with a significantly greater frequency of colocalization after 500 nM αS treatment compared to 100 and 250 nM. Analysis of LC3 via western blot also demonstrated marked increase in LC3 expression after all treatment concentrations, similar to control cells. However, p62 analysis, which when decreased indicates autophagic flux and lysosomal degradation, only differed in apoE ε4/ε4 astrocytes after 500 nM treatment of αS, and not 100 nM or 250 nM treatments. Also, unlike control cells, increasing concentrations of αS resulted in increased cytotoxicity 75% greater than control after 100 nM treatment, 4.8 fold greater after 250 nM and 16.2 fold greater after 500 nM. Perhaps significantly, although increased GFAP+ cells were observed at low levels of αS treatment in control cells, this was only observed in high concentrations of 500 nM αS in apoE ε4/ε4 astrocytes, indicating a possible protective effect in the inflammatory response that was not mobilized in apoE ε4/ε4 astrocytes. The results here indicate that αS colocalizes with lysosomes, causing autophagosome formation, but without induction of autophagic flux except at high concentrations. This was insufficient to protect apoE ε4/ε4 astrocytes from cytotoxicity, which increased exponentially with extracellular αS treatment in all conditions.

T05-068E

Mild uncoupling of astrocyte mitochondria: a strategy to rescue spatial memory deficits in Alzheimer's disease

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Hypometabolism and oxidative stress, along with neuronal death are often associated with the cognitive symptoms of Alzheimer's disease (AD). It has been shown in neuron-astrocyte co-cultures that uncoupling proteins 4 (UCP4) expressed by mitochondria of astrocytes decrease peroxide production, increase their glycolysis and lactate release, enhancing the survival rate of neurons. In parallel, lactate has a non-metabolic effect modulating neural activity. In neurons, lactate has been shown to promote plasticity, gene expression and is a key player for memory formation. We hypothesize that in vivo overexpression of astrocytic mitochondrial UCP4 can provide support to neurons facing AD-associated injuries by increasing lactate production.

Adeno-associated virus (AAV) containing mCherry alone or with UCP4 under the GFAP promoter were stereotaxically injected in the dorsal hippocampus of wild-type (WT) and triple-transgenic (3xTg-AD) mice. We assessed spatial memory in 3 and 7 months old mice by performing a spatial recognition task. Viral construct had no effect on the exploratory behavior of both groups. WT mice, regardless of their age, did not display memory deficits, while 3 and 7 months old 3xTg-AD mice showed spatial memory impairment. Uncoupling astrocyte mitochondria appeared to rescue spatial memory deficits in 7 months old 3xTg-AD mice.

Whole cell patch clamp recordings of subicular neurons involved in spatial coding, of 7 months old 3xTg-AD mice showed alteration of burst frequency and AHP current that was reversed by UCP4 treatment. Overall, astrocytic UCP4 overexpression appears to positively influence AD symptoms both at behavioral and cellular levels.

T05-069E

Deciphering the role of AIM2 in myelin damage and repair in a murine model of MS

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Multiple sclerosis (MS) is a debilitating disease affecting the central nervous system (CNS) caused by damage to myelin, which can lead to neurodegeneration. Myelin damage is caused by immune cells inflicting damage to myelin and oligodendrocytes, the myelin-producing cells in the CNS. There are no treatments available that can therapeutically enhance myelin regeneration and prevent axonal degeneration. Recently, immune mechanisms were shown to promote remyelination in experimental models of MS.

Absent in melanoma (AIM) 2 is a cytosolic dsDNA receptor that can form inflammasomes upon dsDNA sensing and also function independent of the inflammasome complex, such as determining the functional role of T_{reg} in MS. The role of AIM2 in de- and remyelination is not known. This project aims to investigate the function of AIM2 in myelin damage in the CNS.

Using a focal demyelination model, we show that AIM2 is expressed in cells within the demyelinated lesion

including during the myelin regenerating phase. We compared glial cell populations in remyelinating lesions of wild type (WT) and AIM2 deficient animals.

AIM2 did not affect oligodendrocyte progenitor cell (OPC) proliferation nor OPC differentiation after damage. However, AIM2 deficiency decreased microglia/macrophage populations from 7 days after myelin damage compared to WT mice. Conversely, astroglia were increased in the absence of AIM2 at 14 days post injury. This suggests that AIM2 can modulate glial populations after myelin injury. Further investigation will unravel the underlying mechanisms of how AIM2 regulates glial cell populations in the demyelinated lesion and what impact that has on remyelination.

Acknowledgement

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T05-070E

Targeting a signal of glial activation with Semaphorin 4D blocking antibody pepinemab

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Drivers of glial cell activation may represent important targets to preserve normal homeostatic maintenance and modify progression of neurodegenerative pathology. Semaphorin 4D (SEMA4D) is upregulated in neurons in response to stress, and SEMA4D binding to its cognate plexin B receptors regulates microglial activation, survival and differentiation of glial precursor cells, disruption of the neurovascular unit, and astrogliosis with concomitant loss of some normal astrocyte functions. SEMA4D induced downregulation of astrocytic glutamate and glucose transporters, EAAT-2 and Glut-1, while antibody blockade inhibited these effects and reversed deficient glucose uptake in vitro. In vivo antibody treatment normalized neuroinflammatory pathology and ameliorated symptoms in preclinical models of multiple sclerosis, Rett syndrome, Huntington's disease (HD) and Alzheimer's disease (AD).

Pepinemab, a humanized anti-SEMA4D monoclonal antibody, was evaluated in a double-blind, randomized, placebo-controlled Phase 2 study in subjects with HD. Subjects were treated with monthly infusions of pepinemab for at least 18 months and evaluated for safety and clinical parameters including cognition (HD-CAB) and Clinical Global Impression of Change (CGIC). Secondary imaging endpoints included structural MRI to assess brain atrophy and FDG-PET to assess brain glucose metabolism. Pepinemab was well-tolerated and was shown to cross the BBB at the anticipated level. Primary efficacy outcomes did not achieve statistical significance, however trends favored pepinemab., Exploratory analyses demonstrated a treatment benefit in 6/6 components of the HD-CAB cognitive battery, with a significant increase in HD-CAB composite index ($p=0.007$) in subjects with early manifest (EM) HD who received pepinemab treatment. Among EM subjects with somewhat more advanced disease progression, treatment also reduced deteriorating CGIC status ($p=0.04$). Pepinemab treatment reduced brain atrophy (volumetric MRI) and slowed or reversed decline in metabolic activity in 26/26 brain regions, with 15/26 ROI showing a significant positive treatment effect ($p\leq0.05$) in FDG-PET imaging. Reversal of metabolic decline associated with disease progression supports the proposed mechanism of restoring astrocytic metabolic

homeostasis. Further improvements in cognition highlights the critical role for astrocytes and glia contributing to neuronal function and protection in neurodegenerative processes. SIGNAL-HD demonstrated pepinemab's safety and encouraging treatment effects in a clinical study. The mechanism of action is believed to be applicable to diseases exacerbated by inflammatory glial activation. A Phase 1 study in AD is planned.

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T06 | (Energy) Metabolism

T06-001C

Astrocytic mitochondria regulates astrocytes morphogenesis and supports synaptogenesis

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The mechanisms controlling the postnatal maturation of astrocytes play a crucial role in ensuring a correct synaptogenesis. Here we explore the functional significance of mitochondrial activity for post-natal maturation of astrocytes *in vitro* and *in vivo*. We show that postnatal morphogenesis of astrocytes is critically dependent on the transient up-regulation of metabolic regulator PPARγ co-activator 1α (PGC-1α) which is required for determining the highly interconnected functional network of mitochondria and then for providing an efficient bioenergetic supply in the developing astrocytes. The integrity of the elongated mitochondrial network depends on the metabotropic glutamate receptor 5 (mGluR5) via a direct modulation of PGC-1a. We also report that perturbation of mitochondria network and function by ablation of the PGC-1α or of the mGluR5 in the developing astrocytes leads to a delayed morphogenesis of astrocytes and an impairment in the formation and function of neighbouring synapses, whereas genetic re-expression of PGC-1a in astrocytes restores mitochondrial functions and ameliorates both astroglial and neuronal defects. Together with the finding of the development-associated enhancement of mitochondrial function in astrocytes, our results uncover mitochondria activity as a novel mechanism controlling astrocyte morphogenesis and supporting synaptogenesis, thus identifying the mitochondrial function of the developing astrocytes as potential target to ameliorate the formation and function of synapses. Support contributed: *Swiss National Foundation NCCR "Synapsy" (51NF40-158776) and "Transcure" (51NF6240-16) to PB, SNFS (26074366) to PB.*

T06-002C

Deficits in brain energy metabolism in a mouse model for Glut1 Deficiency Syndrome

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Glucose transporter type 1 deficiency syndrome (Glut1DS), also called De Vivo disease, is caused by mutations in *SLC2A1* gene that encodes for glucose transporter type 1 (Glut1). In the brain, Glut1 is predominantly expressed in endothelial cells of the blood brain barrier and astrocyte membranes. In human, this mutation results in deficient glucose transport to the brain and is characterized by early onset epilepsy, complex movement disorders and cognitive impairment.

In order to assess the impact of Glut1 deficiency on brain energy metabolism, we measured glycogen, lactate, and glucose levels in the cerebral cortex and hippocampus of a mouse model for Glut1DS. Glut1DS mice were obtained from the De Vivo laboratory and generated by targeted disruption of the mouse GLUT1 gene. GLUT1+/- mice mimic the major features of the classical phenotype of human Glut1DS (Wang D. et al, 2006). GLUT1+/- and wild type (WT) mice were sacrificed by focused microwave irradiation of the brain. Glycogen, lactate, and glucose levels were measured in the cerebral cortex and hippocampus using standard biochemical assays.

A significant decrease in glucose levels was already observed at 2 weeks of age both in the hippocampus and the cerebral cortex of GLUT1 +/- mice compared to WT mice (-29% and -42%, respectively). In addition to decreased glucose, we found a significant reduction in glycogen concentrations (hippocampus: -29.8%, cerebral cortex: -30.9%) and lactate level (hippocampus: -36.9%, cerebral cortex: -24.7%). In 10-week old mice, a similar decrease in glucose levels was observed in the hippocampus and cerebral cortex of GLUT1 +/- compared to WT mice (-31.9% and -40.8%, respectively). Decreases in glucose levels are accompanied by a significant reduction in cerebral glycogen content (hippocampus: -32.9%, cerebral cortex: -35.3%) and lactate levels (hippocampus: -15.7%, cerebral cortex: -17.8%) in GLUT1 +/- mice compared to WT mice. No difference in glycaemia and lactatemia was observed between GLUT1+/- and WT mice. In addition, there was no significant difference in glycogen, glucose and lactate levels between male and female in GLUT1+/- and WT mice.

Together, these data show that Glut1DS has an early impact on brain energy metabolism not only on glucose levels but also on other important energy substrates including lactate and glycogen. As astrocytes store glycogen and release lactate, an important energy substrate and signaling molecule for neurons, these data suggest that Glut1DS may affect the metabolic cooperation between astrocytes and neurons. Further elucidation of the mechanisms underlying alterations in the astrocyte-neuron metabolic cooperation in GLUT1+/- mice should help to identify novel therapeutic targets for the treatment of De Vivo disease.

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T06-003C

Intracellular lactate originates from glycogen stores in cultured rat astrocytes

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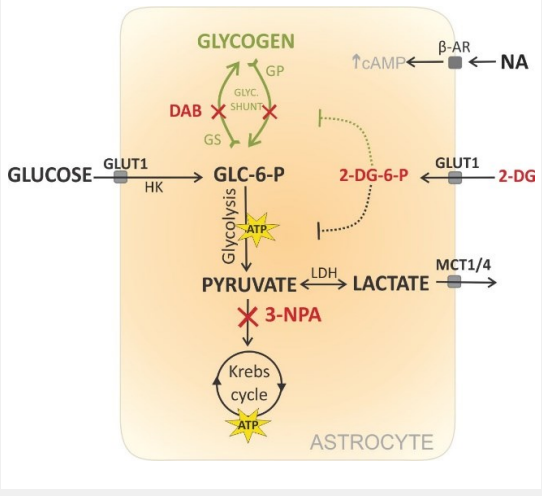
Astrocytes respond to noradrenaline (NA) stimulation with higher rate of aerobic glycolysis - an energy metabolism with end product L-lactate. Biochemical studies showed that astrocytes exhibit a prominent glycogen shunt, in which a portion of uptaken D-glucose molecules is transiently incorporated into glycogen, a buffer and source of D-glucose during increased energy demand.

We measured cytosolic L-lactate ($[lac]_i$) in single astrocytes with the FRET nanosensor Laconic, which we calibrated in astrocytes with perforated plasma membranes. For this we blocked L-lactate dehydrogenase to inhibit the conversion of pyruvate to L-lactate. We generated a calibration curve which was used to estimate $[lac]_i$. Next, we examined whether noradrenaline-induced increase in $[lac]_i$ is influenced by: i) 2-deoxy-D-glucose (2-DG), a molecule that enters the cytosol and inhibits the glycolytic pathway; ii) 1,4-dideoxy-1,4-imino-d-arabinitol (DAB), a potent inhibitor of glycogen phosphorylase and glycogen degradation; and iii) 3-nitropropionic acid (3-NPA), an inhibitor of the Krebs cycle. The results revealed that D-glucose uptake is essential for the NA-induced increase in $[lac]_i$, and that lactate exclusively arises from glycogen degradation, indicating that most, if not all, D-glucose molecules in NA-stimulated cells transit the glycogen shunt during glycolysis. Moreover, under the defined transmembrane D-glucose gradient, the glycolytic intermediates were not only used to produce L-lactate, but also to significantly support oxidative phosphorylation, as demonstrated by an elevation in $[lac]_i$ when Krebs cycle was inhibited.

We found that a large proportion of D-glucose at rest is metabolized in the Krebs cycle, since the resting $[lac]_i$ is only 0.003 mM. This is increased to 0.620 mM after 3-NPA treatment, which blocks the Krebs cycle. This is in line with the view that the oxidative metabolism in mitochondria may metabolize 200x more D-glucose in comparison to D-glucose being metabolized to L-lactate in the glycolytic pathway at rest. However, NA stimulation further increases $[lac]_i$ by a factor of 3. This indicates that substantial aerobic glycolysis occurs only upon adrenergic stimulation of astrocytes. We conclude that oxidative metabolism is important at rest and that in NA-stimulated astrocytes L-lactate production via aerobic glycolysis is an essential energy pathway.



Astrocytic metabolic pathways and targets
D-glucose enters and is either metabolized via glycolysis into pyruvate, or is transitorily incorporated into glycogen. DAB blocks glycogen synthesis and degradation, 3-nitropropionic acid (3-NPA) prevents pyruvate from entering the Krebs cycle. 2-deoxy-D-glucose (2-DG) prevents glycolysis. GP, glycogen phosphorylase; GS, glycogen synthase; HK, hexokinase; LDH, l-lactate dehydrogenase; MCT, monocarboxylate transporter; NA, noradrenaline; β-AR, β-adrenergic receptor.

T06-004C

Modulating microglial metabolism in reducing post-stroke cognitive impairment

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Microglial dysfunction leads to impairment of debris clearance, remyelination, and synapse remodeling, collectively contributing to cognitive decline. New research reveal that some disease-associated microglia (DAM) in neurodegenerative brains present features of elevated phagocytosis, lysosomal functions, and lipid metabolism, which benefit brain repair. However, mechanisms underlying the transformed DAM are not well understood. Intracellular pH (pH_i) is important for regulating aerobic glycolysis and NADPH oxidase activation in proinflammatory microglia. We hypothesize that microglial Na/H exchanger (NHE1) protein, a key pH_i regulator by extruding H^+ (in exchange of Na^+ influx), plays an important role in tuning microglial immune function through modulating microglial metabolisms. We recently showed that selective deletion of microglial *Nhe1* in the *Cx3cr1-CreER$^{+/-}$;Nhe1$^{f/f}$* (cKO) mice switched pro-inflammatory microglia towards restorative polarization after ischemic stroke. Using bulk RNAseq transcriptomic analysis, here we report that the post-stroke cKO microglia displayed stimulation of transcriptomes for genes encoding key rate-limiting enzymes for glycolysis, tricarboxylic acid cycle (TCA), and oxidative phosphorylation (OXPHOS). Moreover, the DAM hallmark genes (*Apoe, Trem2, Spp1,* etc.), as well as genes for phagocytosis (*C1qa*, *Cd68*, etc.), phagolysosomal function (*Ctsb*, *Ctsd, Lamp1*, etc.), and lipid metabolism (*Fdps*, *Agpat5,* etc.) are also increased in the cKO microglia. Pathway analysis revealed significantly elevated metabolism spectrum and LXR/RXR pathway activation for cholesterol export in the cKO microglia. Concurrently, thecKO microglia exhibited increased OXPHOS capacity, reduced cholesterol content, and higher microglial phagocytic activity for synaptic pruning. As a result, the cKO mice presented enhanced oligodendrogenesis and white matter remyelination, which led to significantly improved cognitive functions during post-stroke recovery. Our findings reveal that blocking microglial NHE1 function stimulated glucose immunometabolism and phagocytic functions of DAM for promoting white matter plasticity and synapse remodeling, which collectively contributes to accelerated sensorimotor and cognitive function recovery after brain lesion.

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T06-005C

The electrogenic sodium bicarbonate cotransporter 1 (NBCe1) protects astrocytes from intracellular acidosis during energy failure

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Astrocytes express high levels of the electrogenic sodium bicarbonate cotransporter 1 (NBCe1; NBC), which is a major plasma membrane transporter involved in cellular pH regulation. Owing to its stoichiometry of $1Na^+:2HCO_3^-$, it can operate in inward or outward direction, depending on the respective ion concentrations and the astrocyte's membrane potential. An increase in intracellular Na^+, such as observed during energy failure may thus drive NBCe1 in outward mode, resulting in an export of HCO_3^- and promoting a decrease in intracellular pH.
In the present study, we analyzed the role of NBCe1 in cellular acidification and Na^+ loading during short periods of energy failure employing ratiometric imaging with the ion-sensitive fluorescent indicators BCECF-AM and SBFI-AM in organotypic brain slice cultures and primary astrocyte cultures of the mouse neocortex. Activation of NBCe1 was first probed for by elevation of extracellular K^+ and by monitoring the resulting depolarization-induced alkalinization (DIA), which was accompanied by a decrease in $[Na^+]_i$. When employing a pharmacological blocker of NBCe1 (S0859) or when conducting experiments in slices obtained from NBCe1-deficient mice, both K^+-induced DIA and Na^+ decrease were reduced. Ischemia-like conditions were mimicked by perfusing cells with a glucose-free saline containing the metabolic inhibitors sodium azide (NaN_3, 5 mM) and 2-desoxyglucose (2-DG, 2 mM. Chemical ischemia for 2 minutes resulted in an astrocytic acidosis and a decrease in $[Na^+]_i$. In the presence of S0859 the ischemia-induced acidosis was increased, while the Na^+ load was decreased, suggesting inward operation of astrocytic NBCe1. Similar results were obtained in organotypic brain slices derived from NBCe1-deficient mice.In summary, our results demonstrate that short periods of energy failure result in activation of inwardly-directed NBCe1 in astrocytes. Inward operation of NBCe1 reduces the intracellular acidification of astrocytes during energy failure, presumably exerting a protective role. At the same time, however, NBCe1 activity increases Na^+ influx, promoting astrocyte depolarization. The latter may reduce Na^+-dependent uptake of glutamate and thereby aggravate glutamate-related excitotoxicity.

T06-006D

Effects of Succinylation of Hippocampal Pyruvate Dehydrogenase Complex on Energy Metabolism and Neuronal Plasticity in Mouse Model of Depression

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Studies have shown that mitochondrial energy metabolism plays a crucial role in pathological processes of major depressive disorder (MDD). Succinylation, a newly discovered post-translational modification, mainly occurs in mitochondria and regulates the activities of key enzymes in cellular energy metabolism. Pyruvate dehydrogenase complex (PDHC) is a rate-limiting enzyme during catalyzing the conversion of mitochondrial pyruvate into acetyl coenzyme A (ACA) to regulate glucose metabolism and ATP production. Since almost all ACA in the brain is derived from pyruvate, PDHC dysfunction often leads to mitochondrial energy metabolism in neurological diseases. However, so far, effects of PDHC succinylation on brain energy metabolism and neuronal plasticity in MDD remains unclear. To address these issues, in the present study, chronic unpredictable mild stress (CUMS) was utilized to

generate mouse model of depression. Quantitative succinylation omics analysis confirmed up-regulated PDHC succinylation in hippocampus of depressed mice, while PDH activity was down-regulated. ELISA results showed that the levels of ATP and ACA in hippocampal mitochondria were decreased, while lactic acid (LA) production was increased in depressed mice. The expressions of postsynaptic dense protein 95 (PSD95) and synaptophysin (SYP) in the hippocampus and prefrontal cortex were decreased, as shown by immunohistochemical (IHC) staining and western blot. *In vitro,* dessuccinylase SIRT5 knockdown was accomplished by lentiviral transfection of SH-SY5Y cells. SIRT5 knockdown had no obvious effects on retinoic acid (RA)-induced cell differentiation. Succinylation of mitochondrial PDHC enriched by immunoprecipitation (IP) was up-regulated, while PDH activity was down-regulated in SIRT5-knockdown cells. Besides, production of ATP and ACA as well as expression of PSD95 and SYP were decreased following SIRT5 knockdown. Taken together, the data indicate that up-regulation of PDHC succinylation in the hippocampal neuronal mitochondria could lead to down-regulation of PDH activity, reducing aerobic metabolism of glucose and production of ATP, further affecting neuronal plasticity in depressive model, which could contribute to occurrence and progression of depression.

Acknowledgement

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T06-007D

Glial communication through connexin 43 and energy intake: a possible link.

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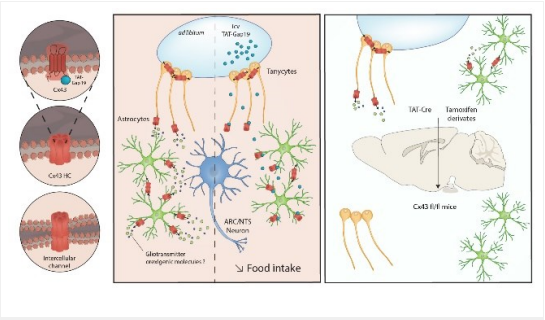
The metabolic syndrome, which comprises obesity and diabetes, is a major public health problem and the comprehension of energy homeostasis control remains an important worldwide issue. The energy balance is finely regulated by the central nervous system, notably through neuronal networks located in the hypothalamus and the dorsal vagal complex (DVC). These structures integrate nutritional, humoral and nervous information from the periphery and in turn adjust energy expenditure and food intake. Glial cells in both the hypothalamus and brainstem interact with neuronal networks to finely tune energy homeostasis. However, the underlying mechanisms and the neuron-glia interactions in the context of food intake control remain to be deciphered.

Astrocytes and tanycytes in the hypothalamus and the DVC strongly express Cx43, a membrane protein belonging to the connexins family. Cx43 hexamers form hydrophilic channels called connexons and pairing of connexons from two adjacent cells can form intercellular channels, gathered in gap junctions, which permit direct cytoplasmic exchanges. Unpaired Cx43 connexons, called hemichannels (Cx43 HCs), also allow the release of neuroactive substances in the extracellular space. Interestingly, levels of hypothalamic Cx43 mRNA and protein were modulated in response to fasting and diet-induced obesity. We assumed this glial communication based on connexins could be involved in the control of energy balance. Thus, we investigated the contribution of glial HCs in rodent's energy balance using a pharmacological blockage of Cx43 HCs opening. We observed a reduction in food intake and a cellular activation of food-intake-dedicated neuronal networks. These results provide the first evidence

indicating that HC activity interferes with the control of food intake and suggest a possible tonic glial delivery of orexigenic molecules via Cx43 HCs. We are now developing models of Cx43 genetic deletion within glial cells of the hypothalamus or the DVC using Cx43 fl/fl mice. To target tanycytes, we perform intraventricular injection of TAT-Cre, a recombinant cell-permeant fusion protein targeting the Cre recombinase to the nucleus. In parallel, we take advantage of tamoxifen derivatives injections in mice harboring, in addition to the Cx43 fl/fl gene, a GFAP-Cre-ERT2 cassette allowing us to pick on mainly astrocytes of these regions. A metabolic characterization is next performed on the different mice models generated.

These advances could help to increase our understanding on the central mechanisms regulating our energy balance and may constitute new therapeutic avenues against overweight, obesity and their co-morbidities.



Contribution of glial connexin 43 in rodent's energy balance
Left: Connexin channels
Midle: pharmacological blockage of Cx43 HCs opening causes a reduction in food intake and a cellular activation of food-intake-dedicated neuronal networks.
Right: Cx43 genetic deletion within glial cells of the hypothalamus or the DVC using Cx43 fl/fl mice

T06-008D

Astrocytic GLUT1 ablation improves systemic glucose metabolism and promotes cognition

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Learning and memory are pivotal processes for survival, enabling the organism to adapt its behavior according to the environment. These adaptive behavioral changes rely on proper brain function and synaptic transmission, which require meeting the energy demands of neurons. Glucose is the main energy source of the brain and thus fuels neuronal activity and memory formation. Glucose supply from the blood to the brain is controlled by the glucose transporter GLUT1, which is highly expressed in astrocytes. Astrocytes are ideally located to control

glucose fluxes between the periphery and the CNS, as they sense and respond to microenvironmental changes, keeping contact with blood vessels and ensheathing synapses (Allaman *et al*., 2011). It is known that ablating GLUT1 at the endothelial cells of the blood-brain barrier (BBB) leads to BBB breakdown, brain glucose hypometabolism and impaired cognition (Winkler *et al*., 2015), but this approach cannot discriminate between the roles of impaired glucose transport and those of altered vasculature and BBB integrity. Such critical question is the focus of the present work, which aims to assess the consequences of astrocyte-specific GLUT1 ablation on astrocytic cellular metabolism, brain and systemic glucose metabolism and cognition.

In our hands, GLUT1-ablated cultured astrocytes showed reduced glucose uptake and glycolysis, although preserving the total ATP production. Unexpectedly, postnatal GLUT1 deletion in glial fibrillary acidic protein (GFAP)-expressing astrocytes ($GLUT1^{\Delta GFAP}$)induced a higher brain ^{18}F-FDG PET signal, indicating enhanced CNS glucose utilization, coupled with a maintenance of CSF glucose levels, without showing BBB breakdown. This also translated into an improved metabolic status of these animals from which high-fat diet (HFD)-fed obese animals especially benefited. Specifically, $GLUT1^{\Delta GFAP}$ mice increased their ability to suppress fasting-induced hyperphagia. Moreover, HFD-fed $GLUT1^{\Delta GFAP}$ mice were more efficient at readjusting systemic glucose levels after hyperglycemia, exhibiting a marked increase in their pancreatic insulin secretion. This effect could be also be linked to the enhanced brown adipose tissue (BAT) thermogenic activity, and reduced BAT adiposity observed in HFD-fed $GLUT1^{\Delta GFAP}$ mice.

Noteworthy, in parallel to the improved systemic homeostasis, $GLUT1^{\Delta GFAP}$ mice were able to adequately perform both spatial (Morris Water Maze) and non-spatial (Novel Object Recognition Test) memory tasks, even outperforming control mice.

Overall, we demonstrate that astrocytic GLUT1 ablation impairs astrocytic glucose availability but enhances brain glucose utilization, reprograms systemic glucose metabolism towards a more efficient glucose-handling phenotype and improves cognitive abilities.

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T06-009D

Stressed astrocytes accumulate lipid droplets

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In pathological states the content of lipid droplets (LDs), the lipid-storage organelles, is increased in the brain, in particularly in glial cells, but rarely in neurons. The (patho) physiology and molecular mechanisms underlying LDs dynamics in astrocytes, glial cells that maintain brain homeostasis, are poorly understood.

We determined the (sub)cellular localization, mobility, and content of LDs under various stress conditions,

characteristic for brain pathologies, by using fluorescence microscopy in combination with Nile Red or BODIPY$^{493/503}$ labeling of LDs, in isolated and brain tissue rat astrocytes. We found that LDs in resting astrocytes are organelles of ~450 nm in diameter localized in the soma and processes in the proximity of endoplasmic reticulum and mitochondria. LDs displayed limited mobility, which was reduced by stimulation- evoked increase in intracellular Ca^{2+} levels and metabolic stress, likely to enhance the LD-organelle interaction. Inhibition of *de novo* LD biogenesis by inhibiting DGAT1 and DGAT2 enzymes reduced astrocyte cell number by ~40%, implying that LD turnover in astrocytes is important for cell survival and/or proliferation. The cellular content of LDs increased by >2- fold in astrocytes exposed to metabolic stress (nutrient deprivation, excess of extracellular FFAs and L-lactate) or hypoxic stress (1% pO_2), indicating accumulation of LDs. A similar increase in LD accumulation was observed upon chronic exposure of astrocytes to noradrenaline, a brain stress response system neuromodulator. The effects of noradrenaline were mediated by activation of β- and α_2- adrenergic receptors and cAMP signals (Figure).
Taken together, our results reveal that noradrenaline, metabolic and hypoxic stress enhance LD accumulation in astrocytes. Accumulation of LDs may be viewed as a support for energy provision in stressed astrocytes, but may also provide neuroprotection against the stress- induced lipotoxicity (1).

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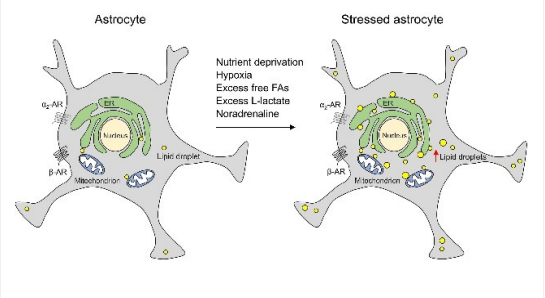

Figure: Stressed astrocytes accumulate LDs. LDs in astrocytes exhibit limited mobility in close proximity to mitochondria and ER. Noradrenaline, a brain stress–response system neuromodulator, metabolic stress, and hypoxic stress increase LD accumulation in astrocytes by increasing the LD number and size. Noradrenaline increases LD accumulation through activation of β- and α_2-ARs. Accumulation of LDs in stressed astrocytes may be viewed as a support for energy provision, but may also provide neuroprotection against the stress-induced lipotoxicity. AR, adrenergic receptor; ER, endoplasmic reticulum; FAs, fatty acids; LD, lipid droplet.

T06-010D

Mitochondria dynamics are altered in chronic experimental autoimmune encephalomyelitis longitudinally and correlate with axon stress

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Postmortem studies of multiple sclerosis (MS) patients demonstrate decreased mitochondrial activity in addition to inflammation throughout the central nervous system. Gait abnormalities in MS patients have been linked to Purkinje cell (PC) demyelination, blebbing axons, and atrophy of dendrites in the cerebellum. Similar changes in PCs are also observed in experimental autoimmune encephalomyelitis (EAE). In addition, axonal degeneration is linked to loss of metabolic support and demyelination. We hypothesize that mitochondrial dysfunction causes axonal degeneration in the context of inflammatory degeneration. To test this hypothesis, cerebellar pathology was investigated longitudinally in EAE disease course from peak disease (day 21) to late disease (day 90). Behavior (walking gait test and rotarod), pathology (immunohistochemistry and Western blot), and mitochondrial function (Seahorse XFp Mito Stress Test) were assessed. From peak disease to late disease, the average EAE clinical score was 2.5. Behavioral tests showed that EAE mice had decreased time on the rotarod and decreased stride length compared to normal. Similarly, increased inflammation and decreased myelination were observed at all timepoints. Mitochondria electron transport chain levels showed no change at peak disease, but chronic EAE showed decreased COXIV, ATP Synthase, mitochondria fusion (Mfn2), and increased mitochondrial fission (Drp1). Mitochondria dysfunction was evident with decreased basal respiration beginning at peak disease and lasting through chronic disease. These data demonstrate that mitochondria dysfunction is evident by peak disease following irreversible axon damage. Future studies will determine whether early treatment with an estrogen receptor b ligand before axon damage occurs can alleviate mitochondria dysfunction and slow neurodegeneration in EAE.

T06-011E

Metabolic reprogramming in phagocytic microglia: from mitochondrial networks to cell function

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Microglial cells are the immune cells of the brain and experts phagocytosing harmful content for parenchymal homeostasis. Apoptotic neurons generated during development in adult neurogenic niches and neurodegenerative conditions leak toxic intracellular content compromising surrounding cells. Microglial phagocytosis avoids the spillover of toxic content and exerts immunomodulatory effects. However, we still do not know the molecular, metabolic, and functional effects of engulfing and degrading an apoptotic cell. To address this question we first performed a global transcriptomic analysis of primary microglia exposed to apoptotic cells and showed that phagocytosis induced changes in metabolic gene expression, suggesting a positive regulation of glycolytic genes and a negative regulation of oxidative phosphorylation genes. We used Seahorse extracellular analyzer to confirm the reduction of mitochondrial metabolism, although we did not detect increased glycolysis. Phagocytosis-induced

metabolic changes were related to a strong remodeling of the mitochondrial network, with fewer and less complex mitochondria. These metabolic and mitochondrial changes compromised cell function, leading to a reduced engulfing capacity of microglia 24 hours after phagocytosis. Furthermore, to study phagocytosis-induced changes *in vivo*, we developed a "superphagocytosis" model using low cranial irradiation (2Gy), which induced newborn cells of the dentate gyrus apoptosis and subsequent increase in microglial phagocytosis. This model offers several advantages to study phagocytosis in vivo, as it considerably increases the number of phagocytotic microglia without damaging them or breaking the blood-brain barrier (BBB). We are currently examining the transcriptome, ultrastructure, and function of phagocytic microglia by performing sc-RNASeq, electron microscopy, and functional analysis. This data will provide us with more information about the complex process of phagocytosis, which we need to understand to use microglia as a therapeutic target in diseases where phagocytosis is impaired.

T06-012E

Identification of Key Processes and Periods in a Model of Human Brain Energy Metabolism by Algorithmic Decomposition with Computational Singular Perturbation

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Energetic considerations play a crucial role in brain function and disease. The study of brain energy metabolism is, however, rendered difficult by a relative lack of experimental tools that can probe the contributions of different intricately connected cell types and processes. In this context, mathematical modelling has a role to play, but typically leads to models, whose complexity reflects that of the underlying biological system, and from which it is difficult to extract biologically actionable information.

Here, an experimentally-calibrated multi-scale model of human brain energy metabolism [1] is analysed using the so-called Computational Singular Perturbation algorithm in order to identify key biological processes and periods, during and after a brain area has been activated. We identify a key role for both oxidative and glycolytic astrocytic metabolism in driving the behaviour of the brain's metabolic circuitry. Neuronal energy metabolism, by opposition, only plays a transient role for the first few seconds after the onset or offset of activation. We additionally identify a key role for the creatine phosphate shuttle in late stages of activation and post-activation. Our approach highlights the importance of glial cells in brain circuits, and introduces a systematic and unbiased methodology to study the dynamics of complex biochemical networks that can be readily scaled to metabolic networks of any size and complexity.

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T06-013E

Tanycytes Control the Hypothalamic Uptake and Metabolic Actions of Liraglutide

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Liraglutide, an anti-diabetic and agonist of the glucagon-like peptide-1 (GLP1) receptor, has recently been approved for the treatment of obesity in individuals with or without type-2 diabetes. Despite its broader metabolic benefits, it is unclear whether liraglutide regulates insulin secretion through specific brain centers or directly via pancreatic beta cells. Here, we demonstrate that liraglutide enters the hypothalamus of mice through specialized ependymoglial cells called tanycytes, bypassing the blood-brain barrier (BBB). Blocking tanycytic transcytosis by cell-specific expression of botulinum neurotoxin impedes the liraglutide-induced activation of hypothalamic neurons and its metabolic effects, including oral glucose-stimulated insulin secretion, mimicking celiac vagotomy. Finally, unlike native GLP1, liraglutide fails to enhance glucose-stimulated insulin-secretion from human islets. Collectively, these data suggest that tanycytes shuttle liraglutide into the brain, where it exerts its anti-obesity effects and induces insulin secretion in response to dietary glucose uptake.

T06-014E

Glial plasticity in the dorsal vagal complex in response to western diet in rodents

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Growing evidence indicates that glial plasticity plays a role in feeding control, by adapting neuronal transmission to metabolic needs. Astroglial morphological changes and microglial activation occurs in response to feeding, in the hypothalamus and in other brain area involved in feeding regulation such as the olfactory bulb. Glial plasticity seems to evolve to glial activation in response to high-fat-high-sugar western diets (WD) consumption generating pro-inflammatory/obesogenic states. There is an abundant glial population in the dorsal vagal complex (DVC), a brainstem area involved in the integration of digestive signals. A thick border of astrocytes delineates the area postrema (AP), a circumventricular organ with permeable blood barrier, from the nucleus of the tractus solitarius (NTS) homing neuronal species involved in satiety, and astrocytes. Microglia are also abundant in the AP and NTS, where pro-inflammatory signals may arrive from the gut in response to dietary load and digestion. However, little is known about the involvement of these glial populations in the satietogenic signals from the DVC.
Our aim was to evaluate astrocytes and microglial changes in response to chronic or repeated episodes of western diet in the DVC of rats or mice.
We have analysed the extent of the astrocyte spreading and the number and morphological phenotype of microglia in the AP and NTS by immunohistochemistry, on two rodent models: rats submitted to a high-fat/high-sugar diet (WD), and mice submitted to several 1-week-episodes of WD. Brainstems were taken after killing, fixed in formalin, and cryostat-cut coronal sections were labelled and analysed for astrocytes (GFAP) and microglia (Iba1).
We found an increase in the astrocyte spreading (GFAP labelled area and thickness) between the AP and the NTS (but not in the astrocytes within the NTS) after one month of WD in rats as well as in mice after the repeated episodes of WD, as compared to control animals. We did not observe any increase in the microglial number or morphology within the AP in WD fed animals whereas we found an increased number of microglial cells in the NTS of WD fed rats as compared to controls. These glial changes were associated with several digestive markers alteration.These results show morphological changes enlarging the astroglial barrier between the AP and NTS in the DVC of rodents receiving a western diet. This was observed after a chronic exposition in rats or repeated expositions in mice, suggesting a persistence of the influence of the diet on the size of the astroglial barrier. Such an astroglial morphological plasticity in the DVC, between the AP and the NTS, may play a role in the adaptation of the satietogenic activity of the neurons to the type of diet.

T06-015E

mitROS signalling controls the onset of CNS myelination

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After birth, partial oxygen pressure rises in the central nervous system (CNS) and induces profound metabolic changes. These changes precede the onset of myelination, a process in which oligodendrocyte precursor cells (OPCs) differentiate and engage with axons in order to invest them with complex multilamellar sheaths. Myelin sheaths enable rapid saltatory signal propagation, synchronised signal conduction, and provide metabolic support to axons. Loss of myelin integrity leads to axonal degeneration and is a hallmark of many neurological conditions.
The complex morphological and functional changes occurring during myelination are likely associated with significant energy demands. Indeed, our previous work characterising glucose-related metabolic pathways during OPC differentiation demonstrated their transition from a low to a high metabolic state. Moreover, inhibition of mitochondria leads to a selective differentiation block.

Investigating how mitochondria control OPC differentiation uncovered the role of reactive oxygen species (ROS) as an important and novel signalling mechanism, with potential therapeutic application.
MitoSox staining demonstrated a significant induction of ROS production as primary rat OPCs differentiate into mature oligodendrocytes. We therefore hypothesised that this increase of ROS may acts a positive signal for OPC differentiation. This was confirmed by treatment of cells with antioxidants (MitoQ and NAC), resulting in reduced OPC differentiation. In contrast, the addition of H_2O_2 to OPCs treated with antioxidants was able to rescue the phenotype and induce OPC differentiation.
Furthermore, we found that ROS are implicated in a feedback loop with mitochondria, upregulating their biogenesis. Most importantly, inducing mitochondrial activity increases the number and the length of myelin sheaths formed by individual oligodendrocytes, while decreasing mitochondrial biogenesis reduces the length and number of internodes of individual cells.
These results provide answers to fundamental questions of oligodendrocyte biology and may have important implications for the development of future treatments.

T06-016E

Aging in the absence of system x_c^- induces metabolic alterations in the hippocampus

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Even though our life expectancy keeps increasing, the current average expected healthy life years is still only around 64. One of the most impactful consequences of aging is the decline of our cognitive functions, which rely on proper hippocampal function. Cells in the aging brain, including the hippocampus, suffer from metabolic dysfunction and the concept of improving cell metabolism to counter the deleterious effects of aging is taking a central role in aging research.

System x_c^- is a glial cystine/glutamate antiporter and mice lacking xCT, the functional subunit of system x_c^-, show lifespan extension with preservation of hippocampal functions (Verbruggen et al., unpublished findings). To investigate the metabolic consequences of the absence of system x_c^- in the aging hippocampus, we performed whole metabolome analysis on hippocampal samples of adult (3m old) and aged (18m old) $xCT^{+/+}$ and $xCT^{-/-}$ mice. Whereas limited differences are seen in the metabolic profile of the hippocampus of adult $xCT^{+/+}$ and $xCT^{-/-}$ mice, random forest classification analysis reveals a distinct metabolome when mice age in the absence of xCT. Pathway enrichment analysis shows indeed key differences in several groups of glycerolipids (plasmalogens, phosphatidylinositols and phosphatidylethanolamines), sphingolipids and one-carbon metabolism (glycine, serine and threonine).

Non-enzymatic post-translational modifications such as glycation and carbamylation, known to negatively affect cellular functions, are also impacted by the absence of xCT. In fact, levels of carboxymethyllysine, a marker of advanced glycation end products, are significantly lower in both adult and aged $xCT^{-/-}$ mice, compared to their age-matched $xCT^{+/+}$ controls. Homocitrulline, as marker of carbamylation, showed an overall aging effect, with only a significant increase in the aged compared to adult $xCT^{+/+}$ hippocampus. Furthermore, the neurotransmitter

acetylcholine -involved in learning and memory - and the sulfate ester of the hormone dehydroepiandrosterone (DHEA-S) -having anti-inflammatory and neurotrophic capacities- were significantly increased with aging; an affect that is supported by the xCT$^{-/-}$ mice. Overall, these results show several metabolic changes in the aged xCT$^{-/-}$ mice which could explain preservation of hippocampal function in the aged brain.

T07 | Extracellular matrix and cell adhesion molecules

T07-001E

The spatial distribution and potential producers of NG2 in the ventral horns with increasing distance from the injury site after spinal contusion

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The idea that the expression of neuron-glial antigen 2 (NG2) proteoglycan is elevated in the area of spinal cord injury (SCI) is not new. Previous research studying NG2 expression has focused on the glial scar and the area immediately adjacent to SCI. However, in order to provide extended axon growth and find targets far removed from the damaged area, it is important to characterize the expression of potential inhibitor molecules in tissue with poorl signs of degeneration adjacent to the injury. In addition, a detailed comparison of the expression and distribution of NG2 proteoglycan has not been yet performed in contusion SCI models.

To identify the spatial distribution and potential producers of NG2 in the ventral horns (VH), a rat contusion SCI model was used. Evaluation of the mean fluorescence intensity of NG2 proteoglycan and population of NG2 expressed cells were defined at different distances (3-5, 6-8 and 10-12 mm) from the epicenter of injury in the caudal direction at 7 and 30 days post injury (dpi).

A quantitative analysis of branched-shaped and pericyte-shaped NG2 expressed cells reviled no differ significantly in these populations which absent or present in very small numbers in VH. The population of $NG2^+/Olig2^+$ cells in the abovementioned zone was also relatively small. The number of $NG2^+/Olig2^+$ cells at 7 dpi and at a distance of 3–5 mm from the SCI epicenter was significantly increased in comparison with intact controls and 30 dpi. However, population of $NG2^+/Olig2^+$ cells did not undergo quantitative changes with increasing distance from the injury site in the caudal direction (6–8 mm and 10–12 mm). The mean fluorescence intensity of NG2 proteoglycan in the VH was highest at 7 dpi near the epicenter (3–5 mm) compared to 10–12 mm distance at the same time point. Although, significant difference in the relative expression of mRNA *Ng2* in comparison with the intact control was found at 30 dpi at 3-5 and 6-8 mm.

Together, these results indicate crucial changes in distribution of NG2 proteoglycan in VH with increasing distance from the injury site in the caudal direction and quantitative shifts in populations of $NG2^+/Olig2^+$ cells predominantly near the epicenter of SCI.

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T07-002E

Hyaluronan accumulates in cortical areas in the ageing brain: interplay with glia

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Beyond neurons and glia, the Central Nervous System (CNS) holds a plastic scaffold known as the extracellular matrix (ECM). In the brain, the interstitial matrix consists mainly of long chains of the glycan polymer hyaluronan. Protein components of the ECM bind to hyaluronan forming a self-assembled matrix that functions as structural framework and signalling hub. Microglia, the never-resting immune cell of the CNS, constantly survey the brain parenchyma, interacting with cells and the surrounding extracellular microenvironment. Astrocytes, the chief regulators in the brain, are the major source of hyaluronan and maintain extracellular homeostasis in the parenchyma. Current data suggest a link between hyaluronan and neuroinflammation, although most results derive from *in vitro* studies and there is scarce information on the glia-matrix interplay *in vivo*, especially in ageing. Here, we report on the interaction between hyaluronan, astrocytes and microglia in the mouse brain of 1, 12 and 18 months. Using high-resolution confocal microscopy and image analysis, we describe structural alterations and changes in the distribution pattern of the hyaluronan matrix in ageing. By analysing the spatial relation between hyaluronan and glia, we gain insight on the turnover of this glycan polymer by astrocytes and microglia, and the effect of its accumulation on the reactive status of these cells. These results shed light on the structure of the extracellular matrix in ageing and its interplay with glial cells, a critical stepping-stone towards exploring this duo in CNS disorders.

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T07-003E

An unexpected role for matrix metalloproteinase 7 upon toxin-induced demyelination

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system in which an unknown trigger leads to inflammation, focal myelin damage and impaired neuronal impulse conduction. The resulting neurological symptoms may improve when demyelinated areas are (partially) remyelinated by surviving oligodendrocytes and/or by the recruitment of oligodendrocyte progenitor cells (OPCs) and their differentiation into remyelinating oligodendrocytes. Unfortunately, as MS progresses, remyelination fails leaving neuronal axons vulnerable to

degeneration resulting in permanent disability. One factor contributing to remyelination failure is the extracellular matrix protein fibronectin. In experimental demyelination-remyelination models, fibronectin is transiently upregulated to stimulate OPC proliferation. However, in chronic MS lesions fibronectin accumulates persisting as aggregates that inhibit OPC differentiation and remyelination. Our previous findings link fibronectin aggregate formation to decreased expression of the enzyme matrix metalloprotease 7 (MMP7), which degrades fibronectin aggregates *in vitro*. In experimental models, MMP7 levels increase upon demyelination and fibronectin is efficiently cleared, but in chronic MS lesions MMP7 is virtually absent, correlating with the persistence of fibronectin aggregates. This leads to the hypothesis that in MS lesions, levels of MMP7 are insufficient resulting in remyelination-inhibiting fibronectin aggregates. To determine whether MMP7 is essential to prevent fibronectin aggregate formation, we investigated remyelination efficiency in MMP7 knockout mice. MMP7 knockout mice and heterozygous controls were fed 0.2% cuprizone-containing chow for either 5 weeks (acute demyelination), normally followed by robust remyelination, or 11 weeks (chronic demyelination). The cuprizone diet led to inflammation and overt loss of (mature) oligodendrocytes in both control and MMP7 knockout mice. However after 5 weeks, when mature oligodendrocyte numbers and myelin basic protein (MBP) expression were still significantly decreased in controls, these parameters were similar to pre-cuprizone levels in MMP7 knockout mice. Furthermore, contrary to our expectations, remyelination in MMP7 knockout mice was equally, if not more efficient upon return to a normal diet. Moreover, in chronic demyelination, the decrease in mature oligodendrocyte numbers was significantly lower in MMP7 knockout mice compared to control, overall pointing to an unexpected negative effect of MMP7 on remyelination efficiency. Elucidating the role of MMP7 in de- and remyelination will improve our understanding of remyelination failure. Ultimately, this knowledge may help to devise strategies to improve remyelination benefiting people with progressive MS.

T07-004E

Atomic force microscopy, a tool for evaluating the regenerative potential of glial cells in brain repair

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Physiological functions and pathologies of the developing brain depend on tight interactions between neurons and different types of glial cells [1]. Previous studies have shown that neuronal growth is substantially impacted by biochemical cues and mechanical stimuli from the surrounding environment [2]. State-of-the-art strategies to reconstruct neural circuits involve stem cell derived products which are used to direct cellular reprogramming and boost effective brain repair [3]. To this end, atomic force microscopy (AFM) is an advanced multiparametric technique that offers key opportunities to simultaneously measure the topographical and mechanical properties of living biological systems [4]. Recent AFM technological advances has made it further possible to resolve dynamic processes on the second and even millisecond scale [5]. In turn, the combination with advanced/customised optics leverages the advantages of immunolabelling techniques for truly correlative microscopy.

We have applied AFM to study the topography of dorsal root ganglia neurons, isolated from a 4-week old B16 mouse. The cells were cultured on laminin-coated substrates 24h post-isolation, and their sensory neurons showed great variation in size and cell bodies. Furthermore, we studied the structure and mechanical properties of astrocytes from a P2 mouse cortex, which enables the correlation between cytoskeletal stiffness and cell topography.

Living cells are constantly interacting with their surroundings, namely by means of membrane turnover, e.g. membrane ruffling, or vesiculation (exo-/endocytosis of metabolites and vesicles). We will demonstrate how such processes can be analyzed at a high temporal resolution during cell spreading and migration in living KPG-7 fibroblasts and CHO cells. We will further show how these processes can be further associated with spatially resolved cytoskeletal reorganization events taking placing under the cell membrane.

External mechanical stress is known to influence cell mechanics in correlation to the differences in actin cytoskeleton dynamics. A crucial aspect of investigating cellular mechanobiology is to go beyond purely elastic models, which do not reflect their complex composition [6], [7]. We have, therefore, performed sine oscillations (up to 1 kHz, amplitude 5-60 nm) in Z while in contact with the sample surface to probe the frequency-dependent response of the cells. This enabled the calculation of the viscoelastic properties, characterised by the dynamic storage and loss modulus distribution in living fibroblast cells.

AFM has a wide range of applications that enable the evaluation of neuronal and astrocyte structure and mechanosensing during development and brain repair.

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T07-005E

New players in glia biology: VPS10P proteins as sorting receptors in astrocytes

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Astrocytes, the most prevalent glial cells in the CNS, have crucial impact on the pathogenesis of virtually all neuropathological conditions including ischemic stroke. However, molecular mechanisms underlying astrocytes activities remain incompletely understood. One important aspect of their function is secretion of specific proteins including cytokines, trophic factors, and ECM components. Moreover, their activity requires proper delivery of certain receptors and adhesion molecules to the cell surface.

The mechanisms of intracellular trafficking and cell surface delivery of transmembrane and soluble proteins in astrocytes have not been extensively studied so far. We propose that these processes might rely on VPS10P domain receptors. The family members SorLA, sortilin and SorCS1-3 are known for their roles in intracellular protein sorting in neuronal cells [1], and our newest data suggest that they can exert similar functions in astrocytes.

Thus, we have recently shown that the sorting receptor SorCS2 is expressed in activated astrocytes in post-ischemic brain where it controls angiogenesis in glial scar region, and we further identified TGFβ1 as a factor inducing SorCS2 expression [2].

Here, we explored the regulatory mechanisms governing VPS10P receptors expression in astrocytes, focusing on the factors driving distinct astrocyte activation modes. We applied unbiased, proteomics-based approach to investigate the repertoire of SorLA, sortilin and SorCS2 ligands in astrocytes. Towards this end, we compared surface proteomes of WT and receptor-deficient astrocytes. With particular interest to tissue remodeling after stroke, we also examined the effects of TGFβ1 on cell surface proteomes and secretomes of wild-type and SorCS2-deficient cells. Our data revealed new potential targets for receptors-dependent sorting in astrocytes. We also identified cell surface and secreted proteins regulated by TGFβ1 in astrocytes, some of which appeared to be SorCS2-dependent. Altogether, these findings unravel mechanisms of the intracellular sorting of astrocytic proteins fundamental to maintenance of functional integrity of the brain.

Acknowledgement

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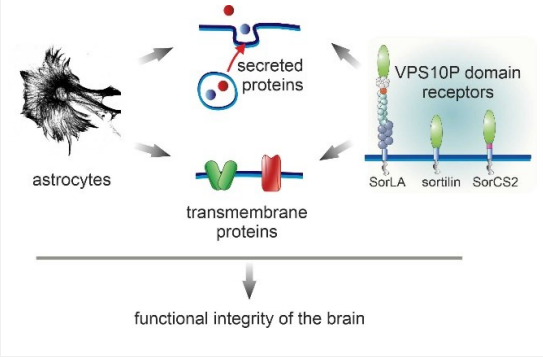

New concept: VPS10P proteins as sorting receptors in astrocytes

T07-006E

Extracellular Matrix Influences Astrocytic Extracellular Vesicle Function in Wound Repair

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Astrocytic responses to injury or infection are known to impact neuropathological outcomes. We had previously determined that astrocyte responses are influenced by the composition of the extracellular matrix (ECM) and this

impacted the wound healing response of astrocytes *in vitro*. Herein, we tested how ECM influences astrocytic EVs and whether EVs may transfer the disparate influences of ECM composition onto naive cells. We report that EVs collected from astrocytes cultured on different ECM substrates are sufficient to convey the influence of ECM experience to direct disparate, but ECM predicated, injury responses in naive astrocytes that recapitulate the influence of the ECM of origin. When compared with wound recovery on control substrates, EVs from ECM-exposed astrocytes elicited accelerated rates of wound recovery that varied based on each ECM (Vitronectin, Laminin, Fibronectin, Tenascin C). This transfer of influence by astrocytic EVs also reflected the experience of the cells to inflammatory stimulation by IL-1b. EVs collected from ECM-exposed astrocyte cultures that were treated with IL-1β were found to affect wound recovery in naive astrocytes in an ECM predicted manner. Because all ECM proteins tested use β1-integrin as a receptor, we also determined that blocking β1-integrin muted the effect of EVs on naive astrocytes indicating that it was the direct response of astrocytes to ECM impacted the qualities of the EVs they release. This provides new information on the importance of culture substrates on astrocytic responses. Moreover, these data suggest that EVs released from astrocytes are altered by ECM, and EVs may be a means by which astroglial responses to injury can alter diverse physiological states in other cells.

Acknowledgement

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T08 | Gene expression and transcription factors

T08-002B

Unravelling the fate determinants of astrocytic identity and their impact in direct neuronal reprogramming

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Astrocytes play essential roles in healthy brain homeostasis and respond to insults by a multitude of cellular, molecular and functional changes. Remarkably, reactive astrocytes express some aspects of the transcriptomic signatures of adult neural stem cells and even show in vitro stem cell hallmarks, thus indicating latent stem cell like properties. Interestingly, astrocytes can also be reprogrammed into neurons both *in vitro* and *in vivo.* With the aim to enhance the neurogenic potential of astrocytes and help their conversion into neurons, we decided to down-regulate key astrocyte fate determinants such as Sox9 and Trps1. While the transcription factor (TF) Sox9 is well known in astrocytes, our genome-wide expression analysis revealed Trps1 as specific for astrocytes, and its function in adult astrocytes remains largely unknown. Using the CRISPR/Cas9 system, we deleted Sox9 and Trps1 and validated the successful deletion first *in vitro*, at DNA, RNA and protein level. To evaluate the role of Sox9 and Trps1 *in vivo*, we injected astrocyte- targeting lentiviral vectors expressing Sox9-Trps1 gRNAs into the adult mouse cortex. Indeed, both proteins are significantly down-regulated already at 7 days post injection (dpi) of the gRNAs in a mouse line expressing Cas9 ubiquitously, and this was still the case at 21dpi. To determine the effect of deleting these factors, we performed patch-seq in acute slice preparations of the cortex injected with control gRNA or Sox9-Trps1-gRNA and performed single cell sequencing by Smart-seq2. Our preliminary analysis suggests changes in gene expression involved in morphology or cytoskeletal architecture, as well as energy production, and confirm the downregulation of several known Sox9 targets.

Together, these data indicate that our knock-out strategy using the CRISPR/Cas9 system is suitable both *in vitro* and *in vivo* to manipulate astrocyte identity: data will be presented on the astrocyte phenotypes elicited by deleting these transcription factors and how this affects direct neuronal reprogramming.

T08-003B

GPCRomics of homeostatic and disease-associated human microglia

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G-protein-coupled receptors (GPCRs) are critical sensors affecting the state of eukaryotic cells. To get systematic insight into the GPCRome of microglia, we analyzed publicly available RNA-sequencing data of bulk and single cells obtained from non-pathological and multiple sclerosis (MS) brains. We identified 17 rhodopsin and adhesion family GPCRs robustly expressed in human microglia form healthy brains, including the homeostasis-associated genes *CX3CR1*, *GPR34*, *GPR183*, *P2RY12*, *P2RY13*, and *ADGRG1*. Expression of these microglial core genes was lost upon culture of isolated cells ex vivo but could be acquired by human induced pluripotent stem cell (iPSC)-derived microglial precursors transplanted into mouse brains. *CXCR4* and *PTGER4* were higher expressed in subcortical white matter compared to cortical grey matter microglia. *ADGRG1* was downregulated in microglia obtained from normal-appearing white and grey matter tissue of MS brains, while microglia from active lesions, obtained early during MS, had downregulated expression of all homeostasis-associated GPCR genes. Of note, single-cell RNA disclosed sequencing upregulated of *CXCR4* expression in a small subset of MS-associated lesional microglia. Functional presence of low levels of CXCR4 on resting human microglia was confirmed using flow cytometry and transwell migration towards SDF-1. Microglia abundantly expressed the down-stream signaling mediator genes *GNAI2* (αi2), *GNAS* (αs), and *GNA13* (α13), the latter particularly in white matter. Drugs against several microglia GPCRs are available, but have not yet been applied in brain diseases. In conclusion, transcriptome profiling allowed us to identify expression of GPCRs that may contribute to brain (patho)physiology and have diagnostic and therapeutic potential in human microglia.

T08-004B

Human fetal microglia acquire homeostatic immune-sensing properties early in development

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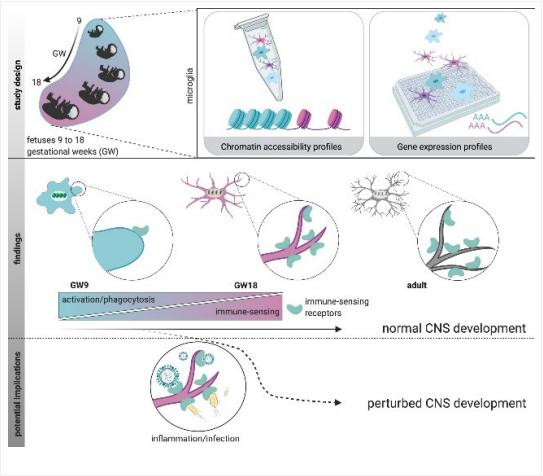
Microglia are the resident immune cells of the central nervous system (CNS). They are important for tissue development and maintenance and are implicated in CNS disease. However, we lack understanding of human fetal microglia development. Here, 15,782 microglia were analysed with single-cell RNA sequencing (scRNAseq) and underlying gene regulatory mechanisms were observed by assay for transposase-accessible chromatin sequencing (ATACseq) in 23 human fetuses ranging from gestational week (GW) 9 to 18. We found that microglia are heterogeneous at all studied GWs and exhibit transcriptional profiles reminiscent of activated/phagocytic microglia. With increasing GW, microglia start to mature and increasingly resemble adult microglia with CNS-surveilling properties. Additionally, chromatin accessibility increases during the investigated developmental period and associated transcriptional networks reflective of adult microglia are identified. Thus, during early fetal development, microglia progress towards a more mature, immune-sensing competent phenotype, which might render the developing human CNS vulnerable to environmental perturbations, such as maternal infection during early pregnancy.



graphical abstract

T08-005B

Analysis of Krüppel like factors in oligodendroglial differentiation and myelin gene expression

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The development of oligodendrocytes, the myelinating glial cells of the central nervous system, is under control of several transcription factors (TFs). One group of TFs involved in oligodendroglial development is the family of zinc finger proteins. These include Znf24, Zfp488 and Sp7 as essential modulators of oligodendroglial differentiation. The krüppel like factors (Klf), a subfamily of zinc finger TFs, additionally seem to play a role in the differentiation of oligodendrocytes. Klf9 has for instance been shown to be an important determinant for remyelination downstream of thyroid hormone, and Klf6 as well is required for CNS myelination as an effector of gp130 signaling. Here, we

take a broader look at occurrence and function of Klf factors in oligodendroglial cells by focusing on the BTEB like subfamily. In particular, we determine whether and how these factors functionally interact with other TFs known to influence oligodendroglial development and myelination such as the Olig proteins, Sox10 and Myrf. These analyses yield further insights into the mechanisms that help to coordinate intrinsic and extrinsic stimuli during oligodendroglial differentiation and the interactions within the underlying transcriptional regulatory network.

T08-006B

Astroglial changes induced by Gfap-dependent expression of a Sox9 transgene

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Both gain-of-function and loss-of-function experiments support the concept that Sox9 expression in neuroepithelial precursor cells (NEPs) of the central nervous system is an essential determinant for glial specification and required for both astrogliogenesis and the production of oligodendroglial cells. As an example, overexpression of Sox9 in NEPs of the developing spinal cord caused an increased and premature generation of oligodendrocyte and astrocyte precursor cells at the expense of neuronal cells in transgenic mice.

To specifically examine the role of Sox9 in astroglial cells after the specification event, we directed expression of a Sox9 transgene to astrocytes using *Gfap* regulatory sequences. Our analysis in the spinal cord showed that absolute numbers and relative percentages for the main neuroectodermal cell populations remained unaffected. However, gene expression within the astroglial population changed substantially following Sox9 overexpression. In the late embryonic spinal cord, a specific subpopulation with defined marker combination became predominant.

By restricting the expression of the Sox9 transgene to a time window before or after birth, effects in the astroglial cell populations are largely suppressed or disappear rapidly. This argues that overall duration and exact time of transgene expression are important for observing the phenotypic alterations. When expressed early enough, the Sox9 transgene seems to keep astroglial cells in an immature state that they can leave again for further maturation after extinction of transgene expression.

T08-007C

RNA localization and local translation in microglial peripheral processes

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Local RNA translation allows the cells to respond fast and efficiently to environmental stimuli. Local translation is especially important in highly polarized cells, such as neurons, because it provides axons and dendrites a means for an accurate response to fast environmental changes. Although most of the work on local protein synthesis in brain cells has been performed in neurons, we now know this phenomenon is not restricted to these cell types. For instance, local translation has been described in peripheral astrocytic processes. In astrocytes local protein synthesis is essential for astrocytes to be involved in synapsis (Sakers et al., 2017). Furthermore, in oligodendrocytes it has been seen that MBP is translated locally in neurodegenerative conditions and during differentiation (Quintela-Lopez et al., 2019). Only recently localized translation in microglia has been established (Vasek et al., 2021), however its role in the pathophysiology of the brain has not been addressed. We propose that local translation in microglia plays fundamental roles in brain function and dysfunction.

We have exposed microglia to different stimuli, like LPS, ATP, Aβ and MCSF, and analysed how they affect local translation in microglial peripheral processes. LPS is the only stimulus inducing changes in local protein synthesis, as well as inducing changes in global RNA localization at the interface between microglial lamellae and filopodia (the leading edge). Additionally, *Actb* transcripts are increased in microglial lamellae and filopodia in response to LPS with LPS.

So far, our results indicate that local protein synthesis might be required for the inflammatory response in microglia cells. We are currently analysing localized translation of *Actb* and other transcripts involved in cell polarity and cytoskeletal rearrangements using puromycilation combined with proximity ligation assay (Puro-PLA)

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T08-008C

H2B monoubiquitination is essential for peripheral myelination and guided by Egr2 in Schwann cells

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Fast saltatory nerve conduction along axons in the vertebrate peripheral nervous system depends on the formation of myelin sheaths by Schwann cells. Coordinated by a complex regulatory network of key transcription factors and epigenetic regulators, Schwann cell differentiation and myelination requires substantial changes in gene expression and goes along with structural alterations in the chromatin architecture. However, little is known on the impact and functional relevance of most of these modifications in Schwann cells. Here, we provide new evidence for a critical function of a specific histone modification, H2B monoubiquitination, during peripheral myelination. Inactivation of the Rnf40-subunit of the responsible Rnf20/Rnf40 E3 ligase in mice revealed that Rnf40-deficient Schwann cells were arrested shortly before myelination onset or generated thin but unstable myelin sheaths that degenerated over time. Eventually, this resulted in progressive peripheral neuropathy characterized by hypomyelination and advancing axonal loss. We found that H2B monoubiquitination does not alter Schwann cell gene expression in a global way, but selectively influences the expression of myelin-related and lipid metabolism genes to ensure their sufficiently high expression while concomitantly controlling the repression of select immaturity genes. This requires Egr2, the master regulator of Schwann cell myelination, to team up with Rnf20/Rnf40 as they act together through specific recruitment of the E3 ligase complex by Egr2 to its target genes. Overall, our data identify H2B monoubiquitination as essential for Schwann cell development and myelination. By showing that main functions of Egr2 rely on the activity of Rnf20/Rnf40 in Schwann cells, these data unravel a new link between central transcription factors and specific histone modifications in the complex underlying regulatory network.

T08-009C

Zinc-finger transcription factor Zfp276 acts as a novel regulator of oligodendroglial differentiation

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High-speed signal transduction in the central nervous system (CNS) requires ensheathment of axons by oligodendrocytes (OLs) with myelin. A complex network of transcription factors is necessary for the timely regulation of OL differentiation and proper myelination. Here, we describe a novel target of the key transcription factor Sox10, the Zinc-finger protein 276 (Zfp276), and its role as a modulator of OL differentiation and myelination. Zfp276 expression is restricted to mature OLs in the CNS, and its expression is regulated by Sox10, which can activate a regulatory region of the *Zfp276* gene in luciferase reporter assays. We used a conditional knockout mouse model in which Zfp276 is deleted from the OL lineage (Zfp276cko) to analyze the effect of Zfp276 deletion in the spinal cord at different developmental stages. Expression of maturation genes and myelin markers was decreased at early postnatal stages in Zfp276cko mice compared to controls, concomitant with increased proliferation of oligodendrocyte precursors (OPCs). Moreover, ultrastructural analysis of the spinal cord revealed fewer myelinated axons and an increased g-ratio in early postnatal Zfp276cko mice compared to controls. To elucidate the mechanism of Zfp276, we performed luciferase reporter assays on regulatory regions of genes associated with maintenance of the OPC stage as well as of genes important for OL maturation and myelination. We could show that Zfp276 inhibits the Sox10-dependent transactivation of OPC related genes, but not of myelin genes. Chromatin immunoprecipitation and gel retardation assays furthermore confirmed that Zfp276 acts by direct

binding to its target genes via its pentameric zinc-finger domains. In conclusion, our data identified Zfp276 as a new transcriptional regulator of OL differentiation and myelination in the CNS, most likely by inhibiting immaturity factors, thereby fine-adjusting the timely onset of myelination.

T08-010C

The Influence of the histone acetylase Tip60 on Schwann Cell Development in mice

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Schwann cells are the myelinating glia of the PNS. It is known for many years that the development and differentiation of peripheral glia depends on transcription factors such as Sox10, Oct6 and Krox20. More recently, chromatin remodeling complexes draw increasing attention, including the Brg1-containing BAF complex and the Chd4-containing NuRD complex. Such complexes consist of multiple subunits and many chromatin modifying factors have not yet been analyzed during Schwann cell development and myelination. After having unraveled the important functions of the ATPase Ep400 as one of the central subunits of the Tip60/Ep400 chromatin remodeling complex during Schwann cell development and differentiation, we now focus our research on Tip60, the second central subunit of the complex. After binding to H3K4me1, this enzyme acetylates histone H4 and histone H2A to allow the subsequent exchange of H2A with its variant H2A.Z and alter gene expression. Besides histone acetylase activity, Tip60 is known to be involved in DNA damage repair, apoptosis, cell signaling and metabolism in various cell types. To analyze important functions of Tip60 in myelinating peripheral glia, we selectively deleted Tip60 in immature Schwann cells by Cre-mediated recombination. Postnatally, Tip60-deficient animals develop signs of hypomyelination and peripheral neuropathy. This is accompanied by a drastic reduction of mature, myelinating Schwann cells and myelinated axons. Reduction of compact myelin causes sciatic nerves to be thinner and appear translucent. Furthermore, loss of Tip60 provokes an increase in cell death. Current studies focus on changes of the transcriptome and the molecular mechanisms of Tip60 in Schwann cell development and myelination.

Acknowledgement

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T08-011C

JAK2-STAT3-dependent molecular signature in reactive astrocytes of the mouse striatum

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Astrocytes have been reported to undergo multiple morphological and functional changes in disease. However, the specific molecular changes unleashed in these reactive cells and their upstream regulators are yet to be fully characterized. The janus kinase (JAK)2/signal transducer and activator of transcription (STAT)3 signaling pathway is a central cascade controlling the reactive response of astrocytes, including in neurodegenerative diseases such as Huntington disease (HD), which primarily affects the striatum. Therefore, we here aimed to define the molecular changes triggered by the JAK2-STAT3 pathway in mouse striatal astrocytes.

We generated adeno-associated vectors (AAV) which target astrocytes specifically and activate the JAK2-STAT3 pathway, by encoding a constitutive active form of JAK2 (AAV-JAK2ca). WT mice were injected in the striatum with AAV-JAK2ca and an AAV-GFP to visualize and sort infected GFP+ astrocytes by fluorescence-activated cell sorting. Control mice were injected with AAV-GFP only. We conducted histological, transcriptomic and proteomic analysis on infected striatal astrocytes.

Histological analysis showed that activation of the JAK2-STAT3 cascade in striatal astrocytes induces morphological changes (increased soma surface and branching index) and the over-expression of GFAP and vimentin, two fundamental gene markers of reactive astrocytes. Microarray analysis of sorted astrocytes showed that the JAK2-STAT3 cascade triggered extensive transcriptional changes with 689 differentially expressed genes between JAK2ca and GFP striatal astrocytes, including the induction of genes linked to inflammation, cytokine signaling and immune reaction. The JAK2-STAT3 pathway also changed the expression of genes involved in proteostasis and energy metabolism. Last, changes in abundance of proteins between JAK2ca and GFP astrocytes was assessed by labeled-free mass spectrometry. By using five software tools, we identified a robust core of 29 proteins, which were also involved in functions related to proteasome, lysosome and oxidative phosphorylation. Ongoing experiments aim to validate the differential expression of candidate genes/proteins in reactive astrocytes in HD murine models and patient brains.

Our multi-omics analysis of the JAK2-STAT3 signature identifies coordinated morphological and molecular changes in striatal reactive astrocytes. These changes could underlie the beneficial effects of JAK2-dependent reactive astrocytes that we observed in HD murine models (see also poster *"Carrillo-de Sauvage. et al."*). A finer knowledge on the molecular signature controlled by specific astrocyte signaling cascades will help outline the different reactive states observed in many diseases of the central nervous system.

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T08-012C

Sensory experience shapes non-neuronal transcriptomic plasticity and cell:cell interactions in primary visual cortex

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Sensory experience shapes the development and plasticity of neural circuits in the brain, yet studies of the mechanisms underlying this process have predominantly focused on neurons. Although evidence suggests that non-neuronal cells (glia) are also modified by sensory input, how diverse glial populations respond to sensory experience remains to be defined. Taking advantage of a robust visual stimulation paradigm, we have begun to systematically characterize the effects of sensory experience on non-neuronal transcription, morphology, and cell:cell interactions in the visual cortex of the mouse. Single-cell RNA-sequencing revealed that light stimulation induces robust transcriptional programs across all neuronal and non-neuronal cell populations surveyed, and that these gene programs are largely cell-type-specific, indicating that experience shapes unique functions of different cell types. A notable deviation is *Slc1a2*, the gene encoding the glutamate reuptake transporter GLT-1 that is critical for clearing excess glutamate from the synaptic cleft. Visual stimulation increases *Slc1a2* expression within all cortical cell types *except* for astrocytes, which decrease *Slc1a2* expression in response to visual stimulation. This finding suggests that most non-neuronal cells may share a sensory-dependent transcriptional mechanism of *Slc1a2* induction allowing them to protect the brain against excitotoxicity in a scenario of acutely increased neurotransmission. Complementing these transcriptomic analyses with immunofluorescence, we have preliminarily observed sensory-dependent changes in endothelial cell organization and neurovascular branching that appear to depend upon the proximity of blood vessels to microglia. On the other hand, a light-dependent decrease in myelination appears to occur independently of microglial mechanisms. Analyses of the effects of sensory experience on other populations of glia are ongoing. Altogether, these findings demonstrate that all classes of non-neuronal cells are responsive to sensory experience at the transcriptional level, and that developmental plasticity is not exclusive to connections between neurons, but also induces the restructuring of functional interactions between glia.

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T08-013D

Transcriptomic Signatures of Human Astrocytes Across Age, Sex, and the Tumor Microenvironment

M. Krawczyk[1], J. Haney[1], C. Caneda[1], R. Khankan[1], S. Reyes[4], J. Chang[1,4], M. Morselli[5], H. Vinters[6,7], A. Wang[4], I. Cobos[8], M. Gandal[1,9], M. Bergsneider[4], W. Kim[4], L. Liau[4,10], W. Yong[11], A. Jalali[12], B. Deneen[12,13], G. Grant[14], G. Mathern[1,4], A. Fallah[4], Y. Zhang[1,2,3]

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Astrocytes are dynamic cells with important roles in brain function and neurological disease. There are notable species differences between human astrocytes and commonly used animal models in gene expression, size, and morphology. However, changes of the molecular attributes of human astrocytes across disease states, sex, and age are largely unknown, which is a barrier in understanding human astrocyte biology and their potential involvement in neurological diseases. To better understand the properties of human astrocytes, we acutely purified astrocytes from the cerebral cortices of over 40 humans across various ages, sexes, and disease states. We performed RNA sequencing to generate transcriptomic profiles of these astrocytes and identified genes associated with these biological variables. Here, we identified a robust transcriptomic signature of human astrocytes in the tumor-surrounding microenvironment. Pathway analysis found upregulation of proliferation processes, along with downregulation of genes involved in ionic homeostasis and synaptic function, suggesting involvement of peri-tumor astrocytes in tumor-associated neural circuit dysfunction. In aging, we also found downregulation of synaptic regulators and upregulation of some markers of astrocyte reactivity, while in maturation we identified changes in ionic transport with implications for calcium signaling. In addition, we identified some of the first transcriptomic evidence of sexual dimorphism in human cortical astrocytes, which has implications for observed sex differences across many neurological disorders. This data provides powerful new insights into human astrocyte biology in several biologically relevant states, that will aid in generating novel testable hypotheses about homeostatic and reactive astrocytes in humans.

Acknowledgement

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T08-014D

The physiopathological role of REST in primary astrocytes

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Neuron-restrictive silencer factor/repressor element 1 (RE1)-silencing transcription factor (NRSF/REST) regulates many genes and signaling pathways involved in neuronal differentiation, synaptic homeostasis and maintenance of normal glial cell functions. REST activity is progressively downregulated in neurons during development, while it is normally expressed in glial cells. Astrocytes are the most abundant glial cells and play an important role in maintaining the integrity of neuronal networks by forming tripartite synapses. The aim of this study was to explore the *in vitro* role of REST in astrocyte functions by using NRSF/REST conditional knockout mice (cKO). We studied the electrophysiological properties of primary cultures of REST-cKO cortical astrocytes by the patch-clamp method. We observed that REST-cKO astrocytes suffer a dramatic reduction of the inward rectifier K^+ current (Kir). We characterized biophysically and pharmacologically this current as mediated by the K^+ channel subtype 4.1 (Kir4.1) that is specifically expressed in astrocytes and mediates the spatial K^+ buffering under physiological conditions. Because a reduction of function of astrocytic Kir4.1 has been shown in a number of neurological diseases including temporal lobe epilepsy, we have studied the firing properties of neurons co-cultured with REST cKO astrocytes. Under these conditions, we observed an overall increase of neuronal excitability. This study adds a new piece to the understanding of the role of REST in glial cells and suggests the development of new therapeutic strategies to cure neuronal hyperexcitability.

T08-015D

Reaction of ependymal cells to spinal cord injury: a potential role for oncostatin pathway and microglial cells

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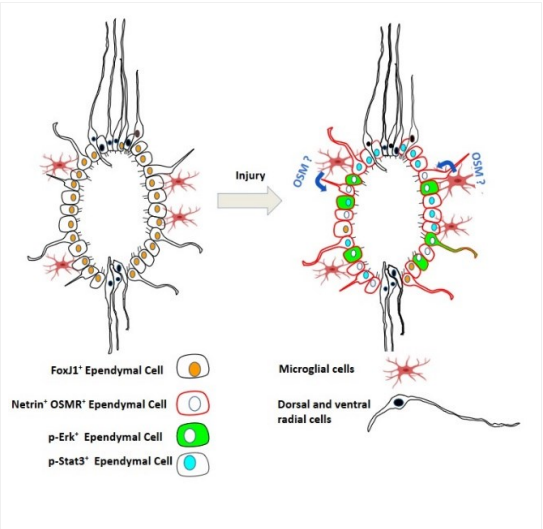
Ependymal cells with stem cell properties reside in the adult spinal cord around the central canal. They rapidly activate and proliferate after spinal cord injury, constituting a source of new cells. They produce neurons and glial cells in lower vertebrates but they mainly generate glial cells in mammals. The mechanisms underlying their activation and their glial-biased differentiation in mammals remain ill-defined. This represents an obstacle to control these cells. We addressed this issue using RNA profiling of ependymal cells before and after injury. We found that these cells activate STAT3 and ERK/MAPK signaling during injury and downregulate cilia-associated genes and FOXJ1, a central transcription factor in ciliogenesis. Conversely, they upregulate 510 genes, six of them more than 20 fold, namely Crym, Ecm1, Ifi202b, Nupr1, Rbp1, Thbs2 and Osmr . OSMR is the receptor for the inflammatory cytokine oncostatin (OSM) and we studied its regulation and role using neurospheres derived from ependymal cells. We found that OSM induces strong OSMR and p-STAT3 expression together with proliferation reduction and astrocytic differentiation. Conversely, production of oligodendrocyte-lineage OLIG1+ cells was reduced. OSM is specifically expressed by microglial cells and was strongly upregulated after injury. We observed microglial cells

apposed to ependymal cells in vivo and co-cultures experiments showed that these cells upregulate OSMR in neurosphere cells. Collectively, these results support the notion that microglial cells and OSMR/OSM pathway regulate ependymal cells in injury. In addition, the generated high throughput data provides a unique molecular resource to study how ependymal cell react to spinal cord lesion.



Adult mouse spinal cord stem cell niche phenotypic modifications observed after injury

T08-016D

Promoting oligodendroglia differentiation from hEScells using an inducible CRISPRa system

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Multiple Sclerosis (MS) is an immune neurodegenerative disorder characterised by demyelination of the brain and the spinal cord, leading to axonal damage. Many immune-centred therapies reduce the inflammation/demyelination during the early stages of MS. However, they do not prevent neurodegeneration during the later stages of MS[1]. Experimental models have shown that remyelination may help avoid neurodegeneration, and therefore this may be beneficial during progressive MS, where remyelination is always inadequate[2]. Current clinical trials to promote remyelination focus on increasing the differentiation of the oligodendrocyte precursor cells (OPCs), into to a myelin-expressing type of oligodendrocyte[3]. However, recent data have shown that human oligodendroglia are heterogeneous, with some expressing many RNA transcripts for myelin production and others few[4]. This leads to

the hypothesis that some subtypes of human oligodendrocytes may be more important for remyelination than others.

To test this hypothesis, we are using oligodendroglia differentiated from human Embryonic Stem cells (hESCs) but this is slow (80+ days), and although heterogeneity is seen, there are relatively few of the oligodendrocytes of interest. Therefore, for functional studies, it is essential to be able to artificially generate large numbers of each subtype of interest.

To do this, we are using a system that combines the efficiency and precision of the CRISPR/Cas9 system with the tunability of the Tet-On system, to artificially differentiate hESCs into oligodendrocyte types. Furthermore, we included mcherry and eCFP in our system for screening purposes, in order to be able to follow and select the cells that contain the inducible machinery.Our preliminary results suggest that, similarly to mouse models where constitutive overexpression of genes specific for the oligodendrocyte differentiation pathway can lead to the generation of oligodendrocytes[5], hESCs can uptake our inducible construct, assessed by detecting the fluorophores. Furthermore, we were able to increase the expression of genes like SOX10 in a tunable manner. Provided that the identity of the cells after the overexpression is similar to the oligodendrocytes obtained using the already established protocols, our data indicate the potential of the hESCs to be used for the generation of different inducible human oligodendrocyte subtypes *in vitro* for functional/screening purposes.

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T08-017D

Analysis of ectopic Sox9 expression in NG2 glia of the adult murine brain

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Repair processes in the diseased or injured adult brain are often similar to processes that are active during ontogenetic development. In the case of demyelinating diseases such as Multiple Sclerosis, repair requires the generation of new oligodendrocytes and myelin by adult NG2 glia. However, adult NG2 glia do not differentiate efficiently compared to their embryonic and early postnatal counterparts. One main difference between NG2 glia in

the early and adult brain concerns the expression of the transcription factor Sox9 that is expressed in early NG2 glia but missing in adult NG2 glia. Therefore, we investigate whether ectopic expression of Sox9 in adult NG2 glia can alter their properties and promote their differentiation capacities. Overexpression of Sox9 in adult NG2 glia indeed causes these glial cells to differentiate more efficiently into myelinating oligodendrocytes. Determining the mechanism of action of Sox9 in adult NG2 glia could therefore help provide future therapies in demyelinating diseases.

T08-018D

Transcriptomic analysis across early postnatal development in healthy and Mecp2 deficient mice reveals aberrant astrocyte maturation in Rett Syndrome mice

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Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the MECP2 gene. The MeCP2 protein is a critical transcriptional regulator in the CNS responsible for neurotypical function and maturation. Persons with RTT experience symptom onset after a period of typical development between 6 to 18 months of age, causing regressions in motor abilities, development of breathing abnormalities, and intellectual impairments requiring life-long care. Mecp2$^{-/y}$ Mouse models similarly recapitulate these symptoms and provide a basis for understanding cellular contribution to disease progression. Previous studies have demonstrated that astrocytes contribute to RTT pathogenesis, but the exact mechanisms are not well understood. Astrocytes are crucial support cells within the CNS, known for their roles in ionic and transmitter homeostasis, injury response, and BBB maintenance. Astrocytes undergo postnatal maturation alongside neurons, indicated by changes in cellular function, and development of a complex morphological phenotype. Surprisingly little is understood regarding astrocyte gene expression during this period of morphological maturation. Understanding astrocyte developmental gene expression patterns may provide insight into neurodevelopmental disorders such as RTT. Using cell-specific isolation of cortical astrocytes, we evaluated the evaluated gene expression in WT and Mecp2$^{-/y}$ mice from early postnatal development through adulthood. We found the highest number of differentially expressed genes (DEGs) during the period of murine astrocyte morphogenesis (P14 – P28, Figure 1) in WT animals. While WT astrocytes have enrichment of pathways associated with cellular morphology and maturation, these pathways are disrupted in Mecp2$^{-/y}$ astrocytes. We also used an AAV construct to drive astrocyte-specific expression of fluorescent membrane markers, determining that Mecp2$^{-/y}$ animals have deficits in astrocyte morphogenesis at the end of this developmental period at P30, but not prior to it at P14 (Figure 2). These results suggest that developmental disruption of astrocyte gene pathways of maturation in RTT may have functional relevance that implicate glial as disease contributors.

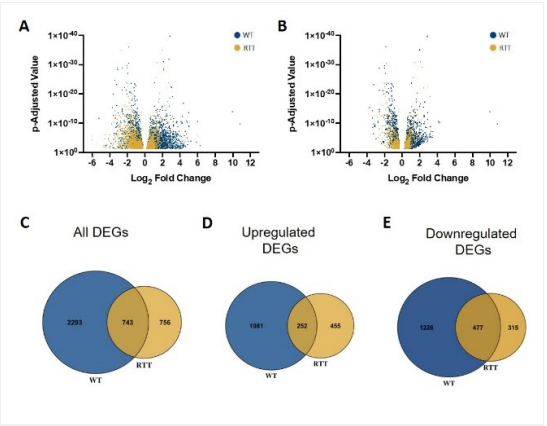

Fig 1. WT v RTT comparison of astrocyte genes across development
A) Volcano plot of all significant DEGS determined in WT (blue) and RTT (yellow) astrocytes between p14 and p28.
B) Plot of DEGS included for analysis in WT and RTT astrocytes between p14 and p28.
C) Venn diagram of all sig. DEGs from WT and RTT astrocytes between p14 and p28 showing DEGs unique to WT (blue, 2293) and RTT (yellow, 756) or common (743) to WT and RTT.
D) Diagram of sig. upregulated from C showing DEGs unique to WT (1081) and RTT (455) or common (252) to WT and RTT.
E) Diagram of sig. downregulated from C showing DEGs uniqe to WT (1226), RTT (315), or common (477).

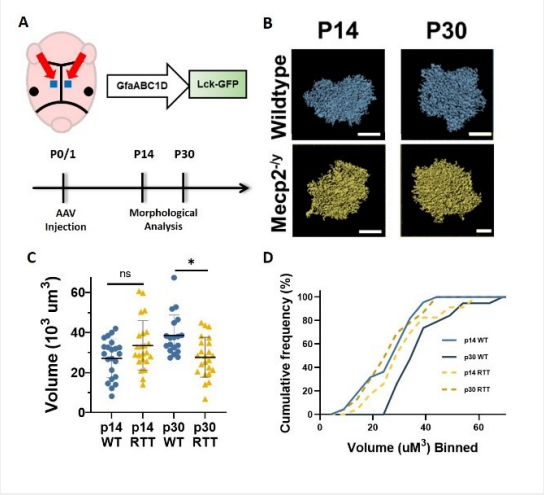

Figure 2. Decreased astrocyte morphological complexity in MeCP2 deficient mice.
A) Schematic of bilateral intracerebroventricular AAV injection for astrocyte-specific expression of membrane-tethered eGFP and morphological analysis timeline of cortical astrocytes in RTT and WT mice.
B) Imaris reconstructions of AAV lck-eGFP+ cortical astrocytes in WT and RTT mice at P14 and P30.
C) WT and RTT cortical astrocytes are not significantly different at P14. P30 RTT astrocytes have ~20% decreased volume compared to WT littermates ($p<0.05$).
D) Cumulative frequency shows left-shift of RTT cKOs towards smaller astrocyte volumes.

T08-020E

Integrated epigenetic and transcriptomic analyses reveal distinct transcriptional programs in mouse cerebellar and cortical astrocytes

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Astrocytes from cortex (CTX) and Bergmann glia of the cerebellum (CB) share basic molecular programs, but also form distinct spatial and functional subtypes. The regulatory basis for this regional diversity has not been systematically investigated. Epigenomic analyses provide important information on cell-specific signatures and the coordination of cell-specific programs on the genome-wide level. We performed a comprehensive genome-wide analysis integrating transcriptome, methylome, and open chromatin data for two astroglia populations isolated from the cortex or cerebellum of young adult mice. We observed that while astroglia populations from the cortex and the cerebellum share a high degree of similarity in both epigenetic and transcriptional programs, they also exhibit strong differences in the chromatin organization and local epigenetic signatures. These differences become obvious in the expression and epigenetic regulation of region-specific transcription factor networks. Most notably, these distinct epigenetically primed networks include transcription factors previously linked to temporal, regional, and spatial control of neurogenesis suggesting the epigenetic maintenance of an early regional developmental priming.

T08-021E

P16-expressing microglial cells with distinct transcriptional profiles accumulate in the aging brain

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Aging tissues accumulate cells positive for the Cyclin Dependent Kinase (CDK)4/6 inhibitor p16 ($p16^{pos}$) which promote multiple age-related pathologies, including neurodegeneration. We show that the number of $p16^{pos}$ cells is significantly increased in the central nervous system (CNS) of 2-year old mice. Bulk RNAseq indicated that genes significantly expressed in $p16^{pos}$ cells were associated with inflammation and phagocytosis. Single-cell RNAseq of CNS cells indicated $p16^{pos}$ cells were primarily microglia, and their accumulation was confirmed in brains of aged humans. Two distinct subpopulations of $p16^{pos}$ microglia were observed with a transcriptome enriched in pro-inflammatory genes that differed from a classical senescence associated gene expression signature, and from the

phenotype of microglia previously described in aged and diseased CNS. Taken together, our study provides evidence for the age-associated accumulation of novel p16-expressing microglia populations that could positively and negatively contribute to brain homeostasis, function and disease.

T08-022E

Transcription factor combinations that define human astrocyte identity encode significant variation of maturity and function

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Increasing evidence indicates that cellular identity can be reduced to the distinct gene regulatory networks controlled by transcription factors. These form stable attractor states in Waddington's landscape. However, redundancy exists in these states as different combinations of transcription factors are able to induce broadly similar cell types. An important question that has not yet been studied in detail is how the functional characteristics of cells induced by distinct sets of transcription factors relate to the cellular identity defined by the attractor state and in particular whether those converge to a singular phenotype. We recently demonstrated that by overcoming gene silencing, it is possible to deterministically reprogram human pluripotent stem cells directly into cell types of various lineages[1]. In the present study we leverage the consistency and precision of our approach to explore different transcription factor combinations encoding astrocyte identity. Analysis of the resulting induced astrocytes (iAs) demonstrated that all transcription factor combinations generated cells with the typical morphology of *in vitro* astrocytes, expressing astrocyte-specific markers. RNA sequencing analysis revealed that the transcriptional profiles of iAs from different transcription factor cassettes, clustered tightly together and displayed similarities with mature human astrocytes, although maturity levels differed between cells. Importantly, we found that transcription factor cassettes induced iAs with distinct differences with regards to their astrocyte-characteristic cell functions, such as their cytokine response to IL-1β and calcium signalling in response to ATP. In conclusion, all our transcription factor combinations were able to induce stable astrocyte-like cells that were morphologically similar but showed subtle differences with respect to their transcriptome. These subtle differences translated into distinct differences with regards to cell function. This insight opens up an opportunity to precision-engineer cells to meet functional requirements, e.g., in the context of therapeutic cell transplantation.

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T08-023E

Development of a test predicting treatment efficacy for multiple sclerosis

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Multiple sclerosis is a chronic autoimmune disease affecting the CNS. It is characterized by inflammatory autoimmune attacks against myelin sheaths leading to demyelination and axonal damage. MS is a complex and heterogenous disease in regard to the histopathological features, clinical course and therapy response of MS patients. To date there is no specific diagnostic test for MS and the treatment choice is based more on risk assessment than the specific needs of the patients. Thus, it is important to define specific molecular biomarkers capable of reflecting the disease activity and facilitate the therapeutic choice among the current treatments for MS.Our group previously characterized a method for predicting the efficacy of anti-cancer drugs. This approach (Fritz et al. Cancers 2020) is based on the determination by RT-qPCR of a restricted molecular signature subjected to successive series of normalizations classifying the molecular targets according to the amplitude of their deregulation. The information revealed by this signature allows the identification of the dominant targets responsible for the development of the tumor thus the major pathways to target to decrease tumor growth. Here, we developed this concept in the context of MS. We generated the EAE-PLP mouse model to collect the brain and spinal cord from animals at different stages of the disease (onset, peak and remission). We produced reference samples including whole brain or spinal cord, sorted oligodendrocytes or microglial and immune cells (CD4, CD8 and CD19). We conducted qPCR analysis in a systematic way for a selection of 25 genes in order to cover important hallmarks of MS such as inflammation, BBB breakdown, gliosis and myelin damage and finally neuroprotective mechanisms. The results show the dynamic expression of these genes in the form of specific signatures for each stage of the disease. The use of the normalization process reveals marked differences compared to the single normalization with the control brain. While identifying new potential therapeutic targets, we were able to monitor MS activity with molecular markers. For example, as a best candidate biomarker for the peak of the disease we identified apelin. We are currently collecting other CNS cells such as astrocytes and neurons to finalize the conception of the test. If successful, this approach will confirm the possibility to develop predictive tools of drug efficacy in CNS diseases.

T08-024E

Distinct amyloid-β and tau-associated microglia profiles in Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent form of dementia and is characterized by abnormal extracellular aggregates of amyloid-β and intraneuronal hyperphosphorylated tau tangles and neuropil threads. Microglia, the tissue-resident macrophages of the central nervous system (CNS), are important for CNS homeostasis and implicated in AD pathology. In amyloid mouse models, a phagocytic/activated microglia phenotype has been identified. How increasing levels of amyloid-β and tau pathology affect human microglia transcriptional profiles is unknown. Here, we performed snRNAseq on 482,472 nuclei from non-demented control brains and AD brains containing only amyloid-β plaques or both amyloid-β plaques and tau pathology. Within the microglia population, distinct expression profiles were identified of which two were AD pathology-associated. The phagocytic/activated AD1-microglia population abundance strongly correlated with tissue amyloid-β load and localized to amyloid-β plaques. The AD2-microglia abundance strongly correlated with tissue phospho-tau load and these microglia were more abundant in samples with overt tau pathology. This full characterization of human disease-associated microglia phenotypes provides new insights in the pathophysiological role of microglia in AD and offers new targets for microglia-state-specific therapeutic strategies.

T09 | Glial-neuronal interactions

T09-001A

Neuronal Subtype-Specific Vulnerability to Demyelination in DRG Neurons

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Peripheral nervous system (PNS) myelin facilitates rapid nerve conduction velocities and provides trophic support to axons. Demyelination of the PNS is the hallmark of several human neuropathies. In addition, demyelination of the PNS is a debilitating result of chemotherapy treatment and diabetes. Demyelination of the PNS dramatically affects sensory neurons and leads to numbness of the limbs, loss of sensation and in some patients, debilitating neuropathic pain. The consequences of mechanical damage of neurons, caused by either crush or axotomy, are well characterized. Strikingly, despite its clinical importance, the effect of PNS demyelination on neuronal gene expression is unknown. To determine the transcriptional response of DRG neurons to demyelination, we characterized the demyelination of the PNS in the PLP-CreERT;ROSA26-eGFP-DTA line. In this line, the expression of the diphtheria toxin A- subunit (DT-A) is prevented by an upstream “lox-stop-lox” (LSL) cassette, and upon PLP-CreERT mediated recombination, the stop sequence is removed, and the expression of DT-A is released, resulting in loss of oligodendrocytes and Schwann cells. We found that in this line the conduction velocity in the sciatic nerve is substantially reduced 21 days post tamoxifen administration (PID21), and full functional recovery of the PNS is achieved three weeks later. In parallel morphological studies we found that the peak of PNS demyelination at PID21 is characterized by appearance of demyelinated and remyelinated large caliber axons, aberrant morphology of Remak bundles, infiltration of phagocytic macrophages, and reduced myelin thickness (increased g-ratio), without axonal loss. To study the effect of this demyelination on neuronal gene expression, we harvested the cell bodies of the sensory neurons that project through the sciatic nerve (DRGs L3-L5) for Single Nucleus RNA-seq (snRNAseq) studies. We sequenced 14,710 nuclei and identified nine naive cell types including glial cells and six different subtypes of sensory neurons (14,096 nuclei). In DRGs derived from the peak of demyelination we identified an additional cluster of nuclei (614 nuclei, ~4% total) that expressed high levels of the known injury-induced genes *Atf3*, *Sox11* and *Sprr1a*. We therefore defined this cluster as “injured”. Cell type annotation of the injured nuclei revealed that the injured nuclei derived specifically from proprioceptive neurons and Aβ rapidly-adapting low-threshold-mechanoreceptor (Aβ RA-LTMR) neurons. Our findings suggest that despite the vast morphological changes in the sciatic nerve upon PNS demyelination, the injury response among DRG neurons is neuronal subtype-specific to proprioceptive and Aβ RA-LTMR neurons, which have the largest myelin sheath.

T09-002A

Alteration in Hepcidin-Ferroportin axis in Activated Microglia Are Linked to Neuronal Cell Death in JEV infection

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Both iron accumulation and microglial activation are recognized as pathological hallmarks underlying the progression of many viral infection diseases including Japanese encephalitis (JE) viral infection; however, their cross-talk is still not clear. The goal of the present study was to analyze the temporal dynamics of iron accumulation and iron transporter protein in the cortical tissue using an intracerebral injection in JE viral infection mice model. We performed a detailed iron accumulation, iron transporter expression analysis as well as neuronal cell death and antioxidant glutathione analysis, through 7 days post-JE viral infection using real-time polymerase chain reaction, biochemical and image analyses. We demonstrated that in the brain, mRNA amount of iron importer gene expression [for e.g.: divalent metal transporter-1 (DMT-1), Zinc transporter 8 (Zip8), Zinc transporter 14 (Zip14), Tf-receptor-1 (TfR1), Tf-receptor-2 (TfR2)] and iron exporter gene expression [for eg; ferroportin-1 (Fpn-1)], increase early after JE viral infection, but the transient upregulation of Fpn-1, rapidly decreases at 7 days post-JE viral infection, which in turn increases the iron level and expression of iron storage protein ferritin (L-ferritin and H-ferritin) and found associated with the increased level of neurodegeneration and reduction in antioxidant glutathione. We further observed that Fpn-1 expression is also significantly down-regulated and related with increased hepcidin level in 7-day ex vivo isolated microglial cells with the increased iron level which is further found associated with upregulation of microglia activation markers TNF-α and IL1β. This study provided in vivo evidence supporting the importance of the microglial hepcidin-ferroportin regulatory axis in the regulation of brain iron metabolism in JE viral infection and implied its potential in the development of neuroinflammation.

Acknowledgement

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T09-003A

Microglia sense neuronal activity indirectly via astrocyte GABA release in the postnatal mouse hippocampus

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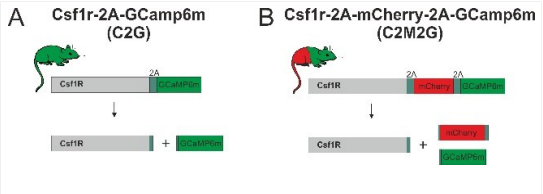
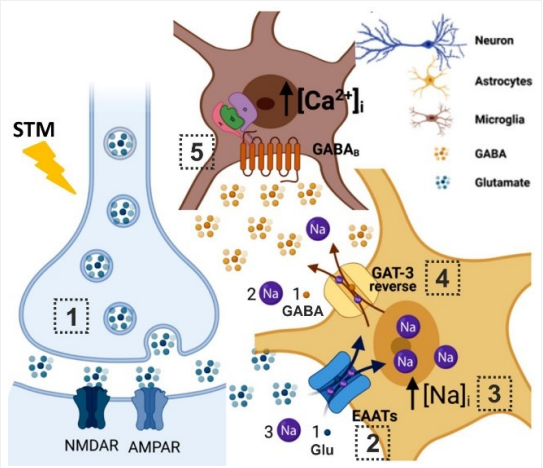
Microglia are the resident macrophages in the central nervous system. Besides the abundant expression of various immune receptors, microglia also express receptors for classical neurotransmitters, as GABA and glutamate, suggesting their potential for sensing synaptic activity. To detect microglial Ca^{2+} in response to neuronal activity, we generated novel transgenic mouse lines which express the fluorescent Ca^{2+} indicator GCaMP6m specifically in microglia. We demonstrate that electrical stimulation of the Schaffer collateral pathway results in transient microglial Ca^{2+} responses at early postnatal, but not adult hippocampus. Microglial responses propagated in a wave-like fashion from the stimulus towards CA1. Preceding the microglial responses, we observed a similar wave-propagation of calcium responses also in astrocytes. Calcium wave propagation in both cell types was sensitive to tetrodoxine but not to ionotropic GABA and glutamate receptor blockers. Blocking astrocytic glutamate uptake or GABA transport abolished microglial responses due to Schaffer collateral stimulation. $GABA_B$ receptors, functionally expressed in microglia, are activated by Schaffer collateral stimulation, but their activation depends on functional glutamate and GABA transport in the astrocytes. Our data therefore suggest that the neuronal activity induced glutamate uptake and release of GABA by astrocytes trigger the activation of $GABA_B$ receptors in microglia. This novel neuron, astrocyte and microglia communication pathway might modulate microglial activity, having in turn critical impact on developing neuronal networks.

Acknowledgement
We are greatful to Andrea Leschke, Bastiaan Pierik, Regina Piske, Nadine Scharek and Maren Wendt for technical assistance and to Alice Buonfiglioli and Dr Niccolò Pampaloni for data discussion. This work was supported by the Helmholtz-Gemeinschaft, Zukunftsthema "Immunology and Inflammation" (ZT-0027) and by the Einstein-Stiftung.



Transgenic strategy used for the microglia-specific GCaMP6m mouse lines
The mouse lines 2A-GCaMP6m (C2G; A) and 2A-mCherry-2A-GCaMP6m (C2M2G; B) were generated via the CRISPR/Cas9 technology and based on C57BL/6 genetic background. For the microglia-specific GCaMP6m expression, a transgene coding for the self-cleaving 2A peptide (2A) and GCaMP6m was inserted at the 3'-end of the endogenous microglia-specific *Csf1r* gene. After the tandem protein translation, the 2A separates the GCaMP6m protein from the Csf1r receptor. A similar strategy was used for the 2A-mCherry-2A-GCaMP6m cassette insertion.



Schematic illustration of the neuron, astrocyte and microglia communication pathway
1.The electrical stimulation of Schaffer collateral pathway lead to presynaptic release of glutamate.
2.Synaptically-released glutamate is uptaken by the astrocytic Na^+-dependent high-affinity glutamate transporters (EAATs).
3.Astrocytic EAATs-mediated glutamate spillover along with the uptake of sodium (Na) might determine an intracellular Na increase.
4.The cytosolic Na rises, increase the probability of the astrocytic GABA transporter GAT-3 to operate in reverse mode leading to astrocytic GABA release.
5.Microglia sense the stimulation-induced astrocytic GABA release via metabotropic GABABR.

T09-004A

Astrocyte calcium signaling in developing sensory domains

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Spontaneous neuronal activity is prevalent in developing sensory systems and characterized by periodic, coordinated activity among neurons that will process information from similar regions of sensory space. This burst firing is conserved between species, resistant to perturbation, and important for both neuron survival and sensory map formation. Astrocytes have been shown to secrete factors that promote synapse maturation and mature in parallel with neurons in the developing brain. However, little is known about the mechanisms that coordinate astrocyte and neuron activity during this critical period. Using *in vivo* calcium imaging in awake neonatal mice we show that bursts of neuronal activity passing through sound processing networks reliably induce calcium transients in astrocytes before the onset of hearing. Astrocyte transients were dependent on high levels of neuronal activity and constrained to regions near active synapses, ensuring close spatial and temporal coordination of neuron and astrocyte activity. Using *in vivo* pharmacology and astrocyte-specific knock-out mouse models, we determined that

astrocyte responses were induced by synergistic activation of two metabotropic glutamate receptors, mGluR5 and mGluR3, which promoted IP3R2-dependent calcium release from intracellular stores. The widespread expression of these two receptors by astrocytes in the developing brain and the prominence of neuronal burst firing in emerging neural networks may help coordinate the maturation of excitatory synapses.

T09-005A

Microglial calcium signaling: Role of neuronal activity and different calcium sensors

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Microglial calcium signaling is an often-dormant component of microglial physiology during in vivo studies of the basal state. However, when neuronal activity changes locally or globally, microglia often respond with a delayed increase in process calcium signaling. Here we explore the role of purine signals and receptors in elevating microglial calcium signaling during hyperactivity, using two-photon imaging in the awake animal and in brain slice. We use multiple genetic approaches, including different GCaMP variants and CreER lines, to explore calcium in microglia. In brain slice, we use pharmacological approaches to explore the key pathways mediating microglial calcium and compliment these studies with genetic knockout lines to identify the key receptors mediating calcium signaling during hyperactivity.

T09-006A

Local translation in perisynaptic and perivascular astrocytic processes – a means to ensure astrocyte molecular and functional polarity?

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Astrocytes are morphologically complex cells, with many ramifications extending towards both blood vessels and neurons. At the vascular interface, astrocytes display perivascular endfeet (PvAP) and regulate important functions such as blood-brain-barrier (BBB) integrity, neurovascular coupling or perivascular homeostasis. At the neuronal interface, perisynaptic astrocytic processes (PAP) regulate synapse formation, gliotransmission, recycling of neurotransmitters and ion homeostasis among other functions. Such morphological and functional polarity is underlined by a molecular polarity, with specific molecules enriched at each interface.

How such polarity is set is unknown. In our laboratory, we hypothesized that mRNA distribution and local translation, one of the most conserved mechanism for cell polarity, sustain astrocyte polarity. We identified polysomal mRNAs enriched in PvAPs and PAPs [1-4]. Our recent findings provided unexpected insights into the PAP's molecular identity - particularly the preferential translation of mRNAs related to iron homeostasis, cytoskeletal dynamics and the translation machinery in these compartments. We also showed that local translation in PAPs was changed upon fear conditioning. Altogether, our results suggested that astrocyte polarity might be sustained by local translation. Moreover, we provided the first evidence of a correlation between fear conditioning and translational changes in PAPs – changes that might be linked to the regulation of synaptic and circuit functions underlying complex behaviors.

Local translation is mediated by *cis*-acting elements that include RNA motifs and secondary structures influencing the binding of *trans*-acting proteins, also known as RNA-binding proteins (RBPs) [5]. mRNAs bind to RBPs to form ribonucleoparticles (RNPs, also referred to as granules) transported along the cytoskeleton as cargo by molecular motor complexes [6]. Although transcriptomic data indicate that RBPs are expressed in astrocytes (sometimes more strongly than in neurons), very few of these proteins have been studied in this context [7]. To address this question, we combined translating ribosome affinity purification with mass spectrometry. The role of identified proteins bound to polysomes in the regulation of local translation is now under investigation.In conclusion, the discovery of local translation in astrocytes leads to address a new generation of questions in particular regarding how astrocytes regulate their high level of polarity in the normal and pathological context. Given the critical functions of astrocytes in the regulation of synaptic and vascular functions, characterization of local translation in these cells might also reveal important and novel aspects of the brain physiology.

Acknowledgement

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T09-007A

Astrocyte Ca^{2+} signalling and adenosine release regulate myelinated axon excitability and conduction speed

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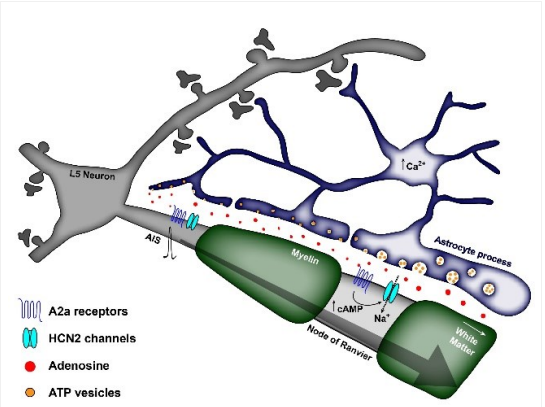
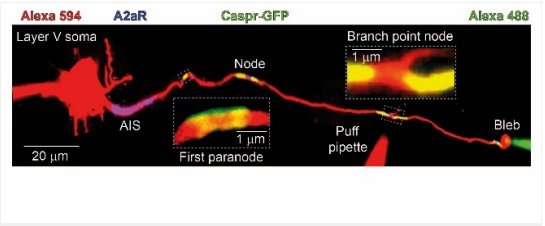
Astrocyte processes regulate synaptic activity and are found close to nodes of Ranvier, but their role in the white matter is unclear. Recently, the axon initial segment (AIS) and the nodes of Ranvier have been proposed to be subject to modulation, but it remains unclear if and how modulation of sites of spike generation is triggered by surrounding cells. Here, we found that adenosine A2a receptors are expressed highly in the AIS and Ranvier nodes of myelinated axons. Single and dual patch-clamping showed that, in layer 5 pyramidal neurons, local activation of A2a receptors in the AIS regulated neuronal excitability, and in the nodes of Ranvier decreased the axonal conduction speed. Moreover, A2a receptor activation induced an increase in cAMP-sensitive I_h inward current, and we found using immunohistochemistry that the hyperpolarization-activated cyclic nucleotide-gated channel subunit HCN2 is present in the AIS and the node of Ranvier. The modulation of myelinated axon excitability by adenosine could be reproduced with computer simulations. Raising astrocyte $[Ca^{2+}]_i$near proximal axons led to a similar change of neuronal excitability that was prevented by blocking A2a receptors and HCN channels. Thus, adenosine-mediated signalling elicited by astrocyte Ca^{2+} activity, whereby G_s-coupled A2a receptors at the AIS and node of Ranvier evoke an inward I_h current, may modulate myelinated axon excitability and conduction speed. This mechanism is expected to modulate information flow when adenosine level rises before sleep, and may explain the white matter disconnection syndromes reported in pathologies such as ischaemia, epilepsy and Alzheimer's disease.

Acknowledgement

Supported by the Wellcome Trust and an EMBO Fellowship



Schematic diagram showing how astrocytes regulate axonal excitability and conduction speed.
Strong triggers of astrocytic $[Ca^{2+}]_i$ rises can lead to Ca^{2+} transients propagating to astrocyte processes contacting myelinated axons. Astrocytic Ca^{2+} activity triggers the release from processes of vesicular ATP, which is converted into adenosine. Adenosine activates $A_{2a}R$ expressed highly in the AIS and nodes of Ranvier. This increases intracellular cAMP levels, thereby opening HCN2 channels. The resulting depolarization leads to two distinct physiological outcomes: in the AIS, this mechanism filters the transmitted output, and in the nodes of Ranvier it reduces the axonal conduction speed.



Experimental setup to study the effect of nodal A2a receptors on axonal conduction speed. The soma and the axon end were patch-clamped with the left (red) and right (green) pipettes, respectively. The A2aR agonist CGS 21680 was puffed at nodes identified by branching of the axons or green signal in Caspr-GFP mice (middle pipette). *Post hoc* immunostaining for A2aR (blue) shows their expression at the axon initial segment and the nodes of Ranvier, and Caspr labelling (green) reveals the first paranode (left inset) marking the start of myelination and allows to detect the nodes of Ranvier along the traced axons. The right inset shows Caspr-labelled paranodes flanking an axon branch.

T09-008A

Astrocyte syncytial isopotentiality shapes neuronal excitability and synaptic transmission in hippocampus

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Gap junctional coupling is known to confer an isopotentiality to astrocyte networks across the brain. However, the impact of syncytial isopotentiality on neuronal circuit function remains unknown. To answer this question, we explored intracellular Ca^{2+} as a potential regulator of astrocyte syncytial isopotentiality. In dual patch recorded pairs of freshly dissociated astrocytes, we found that a *moderate* elevation of Ca^{2+} enhances gap junction coupling, while an *excessive* elevation of Ca^{2+} inhibits gap junction coupling—thus indicating that Ca^{2+} does, indeed, bidirectionally regulate syncytial coupling strength. We next sought to confirm this observation through the mobilization of $[Ca^{2+}]_i$ in transgenic mice with astrocytic expression of Gq-coupled receptors. In mice with astrocytic expression of Gq-MrgA1, *both* potentiation *and* inhibition of gap junctional coupling could be induced by FMRF, an agonist for MrgA1 receptor, at 15 µM and 30 µM, receptively. Notably, these effects were correlated with strengthening and weakening of syncytial isopotentiality detected by whole-cell astrocyte recording with K^+-free/Na^+-containing electrode solution ($[Na^+]_p$), a method recently developed in our laboratory (Ma et al., 2016 Glia). The same effects could be fully recapitulated in the second transgenic mouse line that exhibits astrocytic expression of Gq-DREADD. Finally, we show that in astrocytic Gq-DREADD expressing mice, 10 nM clozapine, a dose sufficient to activate Gq-DREADDs for shutting down syncytial coupling, reduces both neuronal excitability and CA3-CA1 synaptic transmission. Therefore, $[Ca^{2+}]_i$ appears to serve as a key regulator of syncytial isopotentiality, and the open state

of astrocyte syncytial coupling is required for the maintenance of baseline neuronal excitability and synaptic transmission within the brain.

T09-009A

Glial TRPV4 activation by cell swelling suppresses neuronal firing

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We have previously demonstrated that only 30% of astrocytes expressed TRPV4 in brain. Furthermore, activation of astrocytic TRPV4 induced ATP release, and propagated the important signals neighboring astrocytes. In contrast to the brain astrocytes, we found that all of Müller glia expressed TRPV4 in retina (J. Neurosci. 2018). Since Müller glia are functionally relevant to the astrocytes, we hypothesized that the effect of TRPV4 activation on ATP release could be consistent with Müller glia.

Here, we addressed whether the Müller glial TRPV4 activation can induce the ATP release. Using ATP imaging and sniffer patch-clamp methods (in P2X2-expressing HEK293 cells), we demonstrated that the applications of TRPV4 ligands induced strong ATP release from the Müller glia. The strong ATP release was dramatically reduced in the presence of the specific TRPV4 antagonist. To further examined the physiological roles of TRPV4-induced ATP release, we performed patch clamp recordings in acute retinal slices, and recorded the membrane potentials in retinal ganglion cells. The activation of TRPV4 hyper-polarized the membrane potential in the neurons. Our results indicate that the TRPV4 is a key molecule to induce ATP release from Müller glia, and inhibits neuronal excitability in retinal ganglion cells. The TRPV4-mediaded glial ATP release might be an important to processing of vision.

Acknowledgement

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T09-010A

Signals from astrocytes to neurons: exosomes released from astrocytic processes

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In the central nervous system (CNS), exosomes are released into the extracellular space upon fusion of multivesicular bodies (MBs) with the plasma membrane from neurons, astrocytes, microglia and oligodendroglia; they act as non-classical signals in the so-called roamer-type volume transmission, and play multiple roles in both physiological and pathological conditions. While cultured astrocytes have been reported to secrete exosomes, less is known on astrocytes and their ability to release exosomes in neuron-astrocyte networks. In fact, perisynaptic astrocytic processes act as sensors of transmitters modulating the neural activity: they uptake and release gliotransmitters, like glutamate; they also regulate extracellular space volume and coverage of synapses. Indeed, they are specially devoted to bidirectional neuron-astrocyte communication in the tetrapartite synapse and to regulation of synapse plasticity. Here we assess if the astrocytic processes could convey messages through extracellular vesicles (EVs).

Astrocytic processes (gliosomes) were isolated from adult rat cerebral cortex and superfused with standard medium to collect the released EVs. On gliosomes we assessed the purity by IF and WB and the presence of MBs with EM. In gliosomes and EVs, we assessed the presence of exosomal and glial markers by WB, while the vesicle size was measured with DLS. The cell target for exosomes was studied in primary astrocyte-neuron co-culture by IF.

The cortical gliosomes are a purified preparation that presented MBs and expressed the exosomal markers Alix and TSG101, consistent with their ability to release exosomes. The astrocyte-released EVs express the markers for the exosomes and for their parental astrocytic origin. By EM and DLS we observed that the astrocyte-released EVs have the typical cup-shaped appearance and size consistent with exosomes. The astrocyte-released exosomes were also proven positive for neuroglobin (NGB), a protein functioning as neuroprotectant against cell insult. Added in a primary astrocyte-neuron co-culture, the exosomes were able to selectively target neuronal cells and to be internalized by them.

In conclusion, the astrocytic processes, prepared from cortical astrocytes matured in a neuron-astrocyte network, release exosomes, that might participate to signal transmission by targeting near or long-distance cells. The possibility of NGB transfer through astrocyte-released exosomes to neurons would add a mechanism to the potential astrocytic neuroprotectant activity. Notably, the astrocyte-released exosomes maintained the markers proving their parental astrocytic origin. This potentially allows the assessment of the cellular origin of exosomes recovered from body fluids.

T09-011A

Ultrastructural view of internal astrocytic organelles, astrocyte-astrocyte, and astrocyte-synapse contacts within the hippocampus.

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The ultrastructure of astrocyte-astrocyte contacts, the spatial-location selectivity of astrocyte-synapse contacts within an astrocytic domain, and the quantity of mitochondria and vesicles inside astrocytes are unresolved questions. Here, three neighboring hippocampal astrocytes were identified from a P45 *Aldh1l1*-eGFP mouse *prior* to acquisition of EM using serial block-face scanning electron microscopy (SBF-SEM). Using 'inside-out' tracing of astrocytes from nuclear to terminal processes, our reconstructions reveal extensive reflexive, loop-like processes that serve as scaffolds to neurites and give rise to spongiform astrocytic morphology. At the astrocyte-astrocyte interface, a cluster of process-process contacts was identified, which biophysically explains the existence of low inter-astrocytic electrical resistance. We found that synapses uniformly made contact with the entire astrocyte, from soma to terminal processes, and that they can be touched by two neighboring astrocytes. Inside the astrocytes, mitochondrial networks were found to exist throughout the cell—from the nuclear center to peripheral processes until the diameter of the processes become less than 250 nm (termed 'leaflets'). Further, the volume ratio of mitochondria in astrocytes is approximately two times greater than neuronal dendrites and axons, respectively. Lastly, in contrast to densely packed vesicles at the synaptic boutons, vesicle-like structures were scant within astrocytes. Together, these ultrastructural details should expand our understanding of functional astrocyte-astrocyte and astrocyte-neuron interactions.

T09-012A

Nucleus Accumbens Astrocytes Control the Cognitive Impairment Derived from Chronic Exposure to THC

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Drug consumption is a problem that is growing in actual society; therefore it is crucial to fully understand its aftereffects on the normal functioning of the brain. The nucleus Accumbens (NAc) is a key region of the reward system that is implicated in functions related to reward and motivation as well as addictive behaviour. A remarkable feature of the NAc is the integration of motor and limbic information that comes from glutamatergic inputs. Due to the relevance of this communication, it is crucial the maintenance of glutamate homeostasis. Chronic exposure to addictive drugs has shown to imbalance the glutamate homeostasis in NAc, altering plasticity mechanisms such as long-term depression (LTD). Moreover, the existence of modulation of synaptic transmission mediated by activation of cannabinoids receptors (CB1Rs) in astrocytes has been demonstrated (Martin et al 2015, Navarrete & Araque 2010), suggesting that astrocytic CB1Rs are involved in glutamate homeostasis and modulates long-distance communication between neuronal populations (Navarrete & Araque 2008, Navarrete & Araque 2010). However, the functional role of astrocytes in alterations derived from chronic drug exposure is not fully understood.

In this study, we have removed p38α MAPK specifically in NAc astrocytes, which has been described as a key astrocytic molecule in hippocampal LTD (Navarrete et al. 2019). Briefly, we have used the THC-exposure mouse model in p38α MAPK$^{-/-}$ mice with a combination of calcium and glutamate imaging, fiber photometry, molecular, pharmacological and behavioural techniques. First, we analyzed using fiber photometry astrocytic calcium and glutamate activity in NAc *in vivo* after 1mg/kg THC treatment in p38α MAPK$^{-/-}$ and wild-type (wt) animals and we observed that: *1*. THC administration increases astrocytic calcium activity in wt and p38α MAPK$^{-/-}$. *2*. THC administration induces rises glutamate on the surface of astrocytes in wt mice, which is reduced in p38α MAPK$^{-/-}$ mice. *3*. Astrocyte signalling mediated by CB1R induces NMDAR-LTD at NAc and 4. removal of p38α MAPK in NAc astrocytes restore the cognitive impairment derived from THC treatment.

Altogether, our results reveal astrocytes as critical elements for the maintenance of glutamate signalling, with a significant role in drug-consumption related alterations.

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T09-013A

Ischemia-induced changes in intracellular Na^+ and ATP in organotypic tissue slice cultures of the mouse neocortex

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The maintenance of a low intracellular Na^+ concentration by the Na^+/K^+-ATPase (NKA) is a major energy-consuming process in the brain. The reduction in cellular ATP production during an ischemic stroke thus immediately affects NKA function. Weakening of NKA results in an increase in intracellular Na^+, driving cellular depolarization and impairment of Na^+-dependent transport processes. The pathways for Na^+ influx under ischemic conditions are only partially understood.

In the present study, we addressed this question in organotypic tissue slice cultures (OTC) of mouse neocortex using wide-field fluorescence imaging with the sodium-sensitive indicators SBFI and ING-2. In addition, the genetically-encoded FRET-based nanosensor ATeam1.03^{YEMK} was employed to determine changes in cellular ATP. Chemical ischemia was induced by perfusing tissue slices with glucose-free saline, to which the metabolic inhibitors sodium azide (NaN_3, 5 mM) and 2-desoxyglucose (2-DG, 2 mM) were added.

Chemical ischemia for one minute resulted in an increase in astrocytic and neuronal Na^+, accompanied by a decrease in cellular ATP levels. Na^+ loading increased with time in culture; in OTC's kept for 50 *days in vitro* (DIV50), the peak amplitude of ischemia-induced Na^+ transients was almost twice that at DIV7. Moreover, cellular Na^+ increases and decline in ATP were significantly larger at near-physiological temperature (34°C) as compared to room temperature (RT). Pharmacological inhibition of NMDA receptors at 34°C only slightly affected ischemia-induced Na^+ transients in astrocytes, while neuronal Na^+ signals were reduced by ~50%. In both astrocytes and neurons, inhibition of TRPV4-channels resulted in a dampening of changes in intracellular Na^+ as well as in ATP. Combined inhibition of TRPV4-channels and glutamate transporters (GLT-1 and GLAST) further decreased ischemia-induced Na^+ transients in astrocytes, but not in neurons.

Taken together, our results show that metabolic inhibition results in a decline in ATP, which is accompanied by an increase in Na^+ in neurons and astrocytes of organotypic tissue slice cultures of mouse neocortex. The severity of ischemia-induced changes increases with time in culture and with increasing temperature. Opening of TRPV4-channels promotes ischemia-induced Na^+ increases in both cell types. While NMDA receptors represent major pathways for Na^+ influx into neurons, glutamate transporters mediate significant Na^+ loading into astrocytes upon chemical ischemia.

Acknowledgement

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T09-014A

Radial Glial Cells in the optic tectum of zebrafish display synchronized activity events associated with activation of the locus coeruleus.

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There is an increasing interest in understanding the interactions between astrocytes and neurons and how can these modulate sensory processing and behavior. To that end, it is necessary to be able to monitor the spontaneous and sensory-evoked activities of large neuro-glial networks in a behaving animal. In combination with two-photon microscopy and optogenetics, the zebrafish larva model offers the possibility to achieve these aims due to its optical transparency and versatility. Although in zebrafish the bona fide astrocytes have not been described. their Radial Glial cells do not degenerated after development, they show a structural association with neural

processes, express some proteins related to main astrocytes function, and have an intricated structure based on elaborated processes. Together, these findings suggest that radial glia may have further roles in zebrafish similar to mammalian astrocytes. Using transgenic fish and Two-Photon Microscopy, we have found that the calcium activity of the Radial Glial cells in the ventral region of the optic tectum synchronize their calcium activity a few seconds after the end of escape motor behaviors.

The optic tectum is the highest visual center of the larvae brain and it is involved in the detection of surroundings sensory stimuli and generates motor behavior in response. We found that this phenomenon is not dependent on proprioception as it is still observed in paralized larvae.

Optogenetic activation of the locus coeruleus triggers the RGC synchronous events but fails to induce behaviour. In contrast, ablation of the locus coeruleus prevents the RGC synchronous events following escape behaviours.

Therefore, we suggest that the simultaneous activation of the radial cells could be related to alertness or arousal states. To understand the biological relevance of Radial Glial cells synchronization, we performed simultaneous calcium activity recording of neuron and Radial Cells at different layers of the optic tectum and we found no correlations between neural activity and the RGC synchronization. We are now performing additional experiments to shed light on the biological and functional relevance of this phenomenon.

T09-015A

Schwann cells respond to nerve injury with distinct neuroprotective programs.

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Schwann cells (SCs) rapidly sense axon injury and mount unique neuroprotective injury responses. We recently discovered that SCs distal to a nerve injury site reprogram their bioenergetic phenotype in favor of glycolysis to antagonize axonal breakdown [1]. This adaptation to stabilize injured axons is driven by the mTORC1 pathway in SCs. Accordingly, mice with manipulation of glycolytic or mTORC1 components in SCs show altered rates of axonal disintegration upon nerve injury. In parallel to the metabolic remodeling, SCs flanking injured axons are widely known to undergo a transformation to a repair cell phenotype orchestrated by the transcription factor c-Jun [2]. An important component of this conversion is the activation of the autophagy machinery in SCs [3]. This conversion paves the way for efficient regeneration of new axons through the distal nerve stump once disintegrated fibers are cleared away. To determine if the glycolytic shift in SCs is an intrinsic feature of the repair program, we studied glial metabolic injury responses and the rates of axon disintegration in mutant mice lacking c-Jun or key autophagy genes in SCs. We hypothesized differential regulation of these responses and axon disintegration since especially autophagy is tightly regulated by mTORC1 signaling and facilitates glycolysis [4]. Remarkably, we found that c-Jun/autophagy-deficient SCs or nerve stumps show normal glycolytic injury responses. Consequently, the mutants display normal rates of axon disintegration despite compromised myelin clearance in injured nerves. Conversely, mice with inactivation of glycolytic regulators show normal activation of c-Jun and rates of myelin clearance despite accelerated axon disintegration. We also found that c-Jun induction and myelin clearance is mTORC1-independent in SCs, contrary to a recent report [5]. In sum, we reveal that the metabolic reorganization of SCs is mechanistically and functionally distinct from the repair cell program. We propose that the c-Jun-independent glycolytic changes of SCs activated early after injury are aimed at the recovery of injured axons not destined to degeneration. Such setting occurs in disease scenarios in which axons undergo injuries that do not invariably lead to axon destruction. In contrast, the c-Jun-mediated reprogramming of SCs to repair cells is

intended to aid the regeneration of new axons following the disintegration of axon segments unable to recover. However, it is conceivable that sustained glycolytic activity and the release of glycolytic substrates from SCs, perhaps similar to that in *Drosophila* glia [6], may also support axon regeneration [7]. This would suggest the intriguing idea of mechanistic overlap between anti-degenerative and pro-regenerative pathways in SCs.

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T09-016A

Morphological Differences Between Terminal Schwann Cell at the Mouse and Human Neuromuscular Junction

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The neuromuscular junction (NMJ) is the synapse that connects the motor nerves to their target muscle fibres. Glia cells, called terminal Schwann cells (tSCs), are one of the main components of the NMJ. These non-myelinating Schwann cells send processes to completely cover nerve terminals. They play crucial roles in synaptic elimination,

NMJ maintenance, and nerve regeneration after injury. Recent studies revealed significant differences at the cellular and molecular level of rodent and human NMJ, with human NMJs being smaller and more fragmented. However, it is still unknown whether these species-specific differences also extend to tSCs. Here, we have adapted immunohistochemical protocols to facilitate visualisation and comparative morphometric analyses of tSCs at the human and mouse NMJ. We used a-bungarotoxin and anti-S100 to label acetylcholine receptors and tSCs in the peroneus brevis muscle of six adult mice and five humans. A total of 126 mouse NMJs and 151 human NMJs were analysed. Despite the huge difference in NMJ size between mouse and human, we found that both species had a similar number of tSCs per NMJ, an average of ~1.7 tSCs/NMJ. Also, in keeping with differences in overall synaptic size, human tSCs were significantly smaller in size ($p < 0.0001$). More differences between species were also noticed about tSCs cytoplasmic processes and cell body location in relation to the endplate. While both tSCs cell body and cytoplasmic processes tend to be restricted to the endplate in mice, human tSCs tend to have both "synaptic" (~60%) and "non-synaptic" (~40%) nuclei with their cytoplasmic processes extending significantly beyond the synaptic boundaries of the NMJ. Taken together, these findings demonstrate that significant morphological differences between mouse and human tSCs that need to be taken into consideration when translating the findings of both neuromuscular junction biology and pathology from rodents to humans.

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T09-017A

Satellite glia in sympathetic nervous system maintenance and functions

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Satellite glial cells are the predominant glial cell in the sympathetic nervous system, that ensheathe the cell bodies of sympathetic neurons including dendrites and synapses. This places them in a position to regulate synaptic transmission, provide trophic support, and dendritic and synaptic maintenance. However, few studies have investigated the functions of sympathetic satellite glia. We generated tamoxifen-inducible mice, which express diphtheria toxin subunit A (DTA) under the control of brain lipid binding protein-driven Cre, to conditionally ablate satellite glia. We observed a decrease in the number of satellite glia associated with sympathetic neurons and increased apoptosis in sympathetic ganglia with DTA expression in adult mice. Sympathetic neurons in satellite glia-depleted ganglia exhibited deficits in metabolic pathways, specifically mTOR signaling, noradrenaline biosynthesis, neuronal atrophy, and a 25% loss of sympathetic neurons. Together, these results suggest that satellite glia are necessary for metabolism and survival of adult sympathetic neurons. Surprisingly, despite neuronal loss, analyses of autonomic functions such as basal pupil dilation and heart rate suggested that sympathetic tone was elevated in glia-ablated mice, suggesting a role for these glial cells in modulating

sympathetic neuron activity. Potassium buffering is a well-established function of glial cells. Remarkably, conditional loss of the inwardly rectifying potassium channel ($K_{ir}4.1$) in adult satellite glia recapitulated several phenotypes observed with DTA-induced glial ablation including reduced mTOR signaling, neuronal atrophy, and apoptosis.

These results uncover a role for satellite glia cells in modulating neuronal activity and viability, likely via K+ buffering, and identify satellite glia as therapeutic targets in autonomic dysfunctions such as cardiovascular disease and hypertension.

T09-018A

Unique roles of microglial motile processes in phagocytosis of adult-born neurons in the hippocampus

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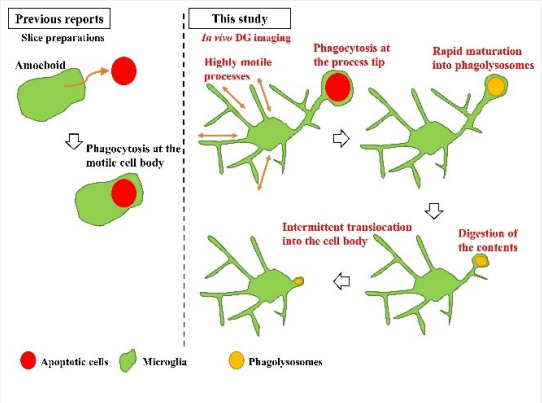
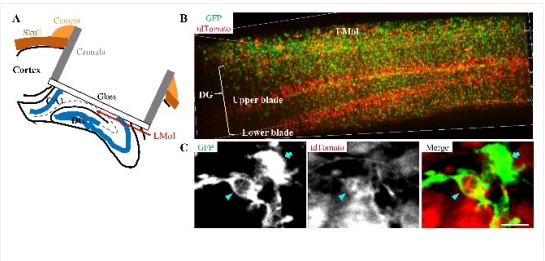
Microglia show phagocytotic activity during brain development and adult neurogenic niches, such as the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG), for the clearance of apoptotic newborn cells. Microglia take ramified morphology with less translocational activity in the adult brain. Nevertheless, they find and eliminate newly generated apoptotic cells. Time-lapse imaging of microglia in slice preparations has provided various information about the phagocytotic process, which often reported microglia of amoeboid morphology, high motility, and phagocytotic activity at the cell body. However, a more physiological process of apoptotic cell clearance in the DG can only be captured by intravital imaging. Here, we performed *in vivo* two-photon laser scanning microscopy and visualized both microglia and newborn cells in the DG to capture microglial phagocytotic events. The surgical procedures and subsequent *in vivo* imaging did not induce pathological activation of microglia in the DG. The imaging resolution was high enough to detect morphological changes in microglial processes. *In vivo* time-lapse imaging and histological analyses revealed the unique phagocytotic events in the adult DG. Highly motile microglial processes survey the surrounding tissue environment for the rapid detection of apoptotic neurons, which were engulfed as entire cell corpses at the tips of the microglial processes. The nascent phagosomes rapidly mature into phagolysosomes with the progressive condensation of the cell corpses, which translocate intermittently toward the microglial cell body. The unique searching, engulfing, and digestion mechanisms of microglia may contribute to the effective selection and removal of a subset of newborn neurons before incorporation into the hippocampal neural circuits. The imaging and analytical methods in this study will be useful for future investigations of microglial function in the DG.



Microglial unique phagocytotic events observed in the adult SGZ.
(Left) Previous reports on microglial phagocytosis in slice preparations: Microglia of amoeboid or less ramified morphology exhibit phagocytotic activity at the cell body.

(Right) *In vivo* DG imaging in this study: With careful control of tissue preservation and minimizing inflammatory responses, DG microglia *in vivo* are stationary with highly motile ramified processes. These microglial processes survey nearby large cell corpses, phagocytize them at their tip, and start corpse digestion inside the processes.



High-resolution in vivo two-photon imaging of the DG for observing the microglial phagocytosis.
(A) Schematic figure of a window implant for DG imaging. CA1 was partially removed to increase the optical resolution for imaging DG, while stratum lacunosum moleculare (LMol) of CA1 was kept intact.

(B) Representative 3D reconstruction image of DG. Green and red fluorescence signals are from GFP-positive microglia and of tdTomato-positive neurons and adult-born neural progenitors.(C) Representative *in vivo* images of microglia in the SGZ phagocytizing tdTomato-positive adult-born cells inside the process (arrowheads) apart from the cell body (arrows). Scale bar, 10 µm.

T09-019A

Astrocyte-mediated phagocytosis in mood and depression-like disorders

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Background:

Major Depressive Disorder (MDD) is a complex recurrent and debilitating psychiatric illness, representing one of the leading causes of disability worldwide. Despite the great economic and social burden of MDD, current pharmacotherapeutic strategies are still lacking efficacy, displaying partial or no response rates to the treatment [1, 2]. The heterogeneity of the clinical manifestations, as well as the different responses to therapies, have made it difficult to get a clear understanding of the disorder etiopathogenesis, emphasizing the relevance of identifying alternative disease trajectories. In the past 20 years, many studies have shed light on the roles of glial cells in brain processes [2]. Accordingly, glial pathology has been described in post-mortem brains of neuropsychiatric patients. Among the three glial-cell types, astrocytes are the most often described source of glial pathology in MDD.

Astrocytes are non-professional phagocytes, which engulf synapses to favour the remodelling of neuronal circuits. MDD displays a disrupted synaptic communication and neuronal connectivity, which both suggest a putative role for astrocytes in its pathogenesis [2]. Astrocytes are an integral part of the synapse and control many aspects of synapse functioning, including the proper recognition, engulfment and degradation of redundant synapses through the multiple EGF-like domain protein 10 (MEGF10) pathway [3]. Interestingly, mice lacking MEGF10 in astrocytes display about 50% reduction in their relative engulfment ability, supporting a strong astrocyte-dependent contribution to the refinement of neuronal synaptic contacts [3]. Previous work from our lab showed that antidepressant drugs (ADs) promote synaptic elimination in primary astrocyte-neuron co-cultures and in the adult rat prefrontal cortex, but not in single neuronal cultures. This event was accompanied by an increase of MEGF10 phagocytic protein levels in astrocytes.

Therefore, a malfunction in the MEGF10 pathway might represent a possible cause for a depressive-like phenotype, leading to the disruption of the proper balance between synapse formation and elimination, a characteristic feature of several neuropsychiatric disorders [4].

Objective: Our aim was to evaluate whether a dysfunctional MEGF10 phagocytic pathway might underlie the neurobiology of depression and may represent a potential novel candidate to develop alternative strategy options for the treatment of MDD.

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T09-020A

Activation of DREADDed astrocytes attenuates pharmacoresistant spontaneous seizures in a chronic epilepsy model

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Astrocytes have long been undervalued in the search for epilepsy treatments, but their crucial importance as active partners in synaptic transmission and regulating neuronal hyperexcitability is now acknowledged (1). Through calcium-dependent gliotransmitter release, astrocytes are capable of activating neighboring neurons and favoring synchronized neuronal activation (2,3). Increased astrocytic calcium transients were found, not only during epileptiform activity (4), but interestingly, preceding epileptic activity *in vivo* (5). These observations indicate that specific targeting of astrocytes and their intracellular signaling mechanisms, may be a viable strategy for developing novel therapies to counter epileptic seizures.

We used chemogenetic modulation of astrocytes for selective modification of astrocyte signaling in the intrahippocampal kainic acid mouse model of pharmacoresistant temporal lobe epilepsy (TLE). This approach combines localized gene therapy (adeno-associated viral (AAV) vectors) and classical pharmacology (using a designer drug such as clozapine-N-oxide (CNO)) to target the astrocytes in the epileptic focus specifically. Astrocyte-specific expression of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) is obtained via injection of AAV vectors under the GFAP promotor in mouse hippocampus: AAV8-GFAP-hM3Dq-mCherry or AAV8-GFAP-hM4Di-mCherry respectively driving expression of Gq-coupled DREADDs (hM3Dq) or Gi-coupled DREADDs (hM4Di). A group of mice with a control vector, lacking the DREADD construct, is used in every experiment for allowing appropriate comparisons. Adequate viral transfection was obtained and DREADDs were solely expressed on astrocytes. We noted a significant increase in c-Fos expression in astrocytes in both non-epileptic and epileptic mice transfected with hM3Dq or hM4Di DREADDs following administration of CNO, showing that even in reactive astrocytes intracellular signaling can be modulated. Most interestingly, chemogenetic manipulation of astrocytes via both Gq- or Gi-coupled DREADDs attenuated spontaneous recurrent seizures in our epileptic mice, suggesting that modulation of signaling in reactive hippocampal astrocytes in a clinically relevant mouse model for TLE can affect seizure burden.

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T09-021B

Generation of human brain spheroids comprising multiple neuroectoderm- and mesoderm-derived cell types

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Three-dimensional (3D) human brain spheroids are instrumental to study central nervous system (CNS) development and (mis)functioning. Yet, in current spheroid models the variety of brain cell types is limited, while a combined set of multiple neuroectoderm- and mesoderm- derived cell types in human spheroids would allow for a more integrated exploration of CNS (disease) mechanisms. We here report a protocol to generate H9 human embryonic stem cell line-derived cortical spheroids (hCS) comprising (1) neuroectoderm-derived neural progenitors, mature excitatory and inhibitory neurons, astrocytes and pre-myelinating oligodendrocyte (precursor) cells as well as (2) mesoderm-derived resting-state and pro-inflammatory microglia and endothelial cells. The (micro)glial cells generated innately in our hCS model were inducible by a variety of cellular stressors. Transcriptome analysis suggests that these multi-cell-type hCS resemble dorsal cortical rather than inferior regions of the human midgestational (weeks 19-26) fetal brain. Thus, our protocol provides a 3D human brain cell model containing a wide variety of not only neuroectoderm- but also mesoderm-derived cell types, furnishing a versatile platform for comprehensive examination of intercellular CNS communication and neurodegenerative disease mechanisms.

T09-022B

Developing Human Pluripotent Stem Cell-Based Cerebral Organoids with a Controllable Microglia Ratio for Modeling Brain Development and Pathology

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Microglia, as brain-resident macrophages, play critical roles in brain development, homeostasis, and disease. Microglia in animal models cannot accurately model the properties of human microglia due to notable transcriptomic and functional differences between human and other animal microglia. Efficient generation of microglia from human pluripotent stem cells (hPSCs) provides unprecedented opportunities to study the function and behavior of human microglia. Particularly, incorporating hPSCs-derived microglia into brain organoids facilitates their development in a 3-dimensional context, mimicking the brain environment. However, an optimized method that integrates an appropriate amount of microglia into brain organoids at a proper time point, resembling in vivo brain development, is still lacking. Here, we report the development of a new brain region-specific, microglia-containing organoid model by co-culturing hPSCs-derived primitive neural progenitor cells (pNPCs) and primitive macrophage progenitors (PMPs). In these organoids, hPSCs-derived pNPCs and PMPs interact with each other

and develop into functional neurons, astroglia, and microglia, respectively. Importantly, the numbers of human microglia in the organoids can be controlled, resulting in a cell-type ratio similar to that seen in the human brain. Using super-resolution microscopy, we demonstrate that these human microglia are able to phagocytize neural progenitor cells (NPCs) and dead cells, as well as to prune synapses at different developmental stages of the organoids. Furthermore, these human microglia respond to Zika virus infection of the organoids, as indicated by amoeboid-like morphology, increased expression of gene transcripts encoding inflammatory cytokines, and excessive pruning of synaptic materials. Together, our findings establish a new microglia-containing brain organoid model that will serve to study human microglial function in a variety of neurological disorders.

T09-023B

Different parameters of spatiotemporal calcium activity in cortical astrocytes are linked to different phases of mouse running

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Numerous reports linked astrocytic calcium dynamics to the range of brain states (sleep, wakefulness, brain perfusion etc.). However, in most cases very simple description such as an increase or decrease in activity was provided. Meanwhile, astrocytic calcium events (temporarily and spatially restricted calcium elevations in the astrocytic network) can be characterized by several parameters, including their areas, durations, spatial density etc. Therefore, we investigated how different spatiotemporal properties of astrocytic calcium activity change depending on the speed of the mouse running in the open field test.

Genetically encoded calcium indicator GCaMP6f was used for calcium signal visualization in 2-3 month-old male C57BL/6 mice. The AAV5 pZac2.1 gfaABC1D-cyto-GCaMP6f was stereotaxically injected with a glass micropipette through round-shaped craniotomy over the primary somatosensory cortex, which was then closed with a cover glass. The mice were placed into a carbon fiber cage and head-fixed using a Mobile HomeCage (MHC, Neurotar, Finland). The MHC is a flat-floored air-lifted platform that allows animals to move the cage with their paws. After training sessions, astrocytic calcium activity was imaged with a two-photon microscope (Femtonics, Hungary) in awake head-fixed mice. Three subsequent imaging sessions for 10 minutes with up to 10-minute rest periods between sets were performed in each mouse. Calcium imaging and animal locomotion tracking based on magnetic sensors were done simultaneously.The locomotion of the animal was accompanied by increased astrocytic calcium activity. When the animal was not moving, scattered localized calcium events at the subcellular level were observed. Individual episodes of locomotion were accompanied by massive activation of the astrocytes in the field of view, while the end of the motion episode was followed by a gradual decline in astrocytic calcium activity. Calcium elevation was due to an increase in both the sizes of calcium events and their density. Interestingly, these two parameters changed independently and were linked to different phases of running episode: enlargement of calcium events transiently occurred at the beginning of running, while the density of calcium events correlated with running speed. Thus, we provide the first evidence that different parameters of spatiotemporal calcium activity in the astrocytic network are responsible for different components of a behavioral incident.

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T09-024B

Molecular mechanism of phosphatidylserine exposure during developmental synaptic pruning

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Developmental synaptic remodeling is essential for precise neural circuitry. Errors in this process have been linked to neurodevelopmental disorders such as autism and schizophrenia. Recent studies show that focally exposed phosphatidylserine (PS) on synapses acts as an “eat me” signal to mediate synaptic pruning. However, the molecular mechanism underlying PS exposure at synapses remains elusive. Mining published retinal ganglion cell (RGC) single cell RNA sequencing data reveals that *Cdc50a*, a gene encoding a chaperone of phospholipid flippases, is the most ubiquitously expressed gene in RGCs compared to related flippase genes. Furthermore, we show that CDC50A is present at synapses. Synaptic inactivity leads to the downregulation of *Cdc50a*. Finally, *Cdc50a* knockdown causes PS exposure at synapses and subsequent aberrant synaptic pruning by microglia *in vivo*. Taken together, our data support that CDC50A functions as a key regulator in microglial-mediated synaptic pruning by focally exposing PS at synapses.

T09-025B

Astrocytes in the ventromedial hypothalamus modulate neuronal activity to regulate anxiety-like behavior through NMDA receptor

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Ventromedial hypothalamus (VMH) plays an important role in regulating chronic stress-induced anxiety-like behavior and energy metabolism disorders. Previous study indicated that astrocytes in other brain regions participate in the regulation of anxiety-like behavior and metabolism through the interactions with surrounding neurons. However, whether astrocytes in VMH could affect neuronal activity and therefore modulate responses to

chronic stress remain elusive. In this study, we first used *c-fos* staining to find that VMH astrocytes were activated after chronic stress, suggesting a functional association between astrocytes activity and chronic stress induced-physiological alterations. Pharmacogenetic activation of astrocytes in VMH resulted in anxiolytic effects, while inhibition caused opposite effects. To dissect the underlying neural mechanism, we applied patch clamp recording and optogenetics manipulation, and demonstrated that optogenetically activating VMH astrocytes induced depolarization in neurons. This depolarization was significantly diminished by administration of N-methyl-D-aspartic acid (NMDA) receptor blocker, but not alpha-amino-3-hydroxy-5-methyl-4 -isoxazole propionic acid (AMPA) receptor blocker. Collectively, our study figured out that VMH astrocytes could affect the function of NMDA receptor on neurons to regulate anxiety-like behavior in mice. Further efforts should focus on exploring the mechanism underlying the regulation of NMDA receptor function by VMH astrocytes.

Acknowledgement

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T09-026B

Oligodendroglia regulate structural and functional neuronal plasticity in the mammalian cortex

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Developmental myelination is a protracted process in the mammalian brain, occurring postnatally in mice and continuing through the first three decades of life in humans. One theory for why oligodendrocytes mature so slowly posits that myelination may stabilize neuronal circuits and temper neuronal plasticity as animals age. We tested this hypothesis in the visual cortex, which has a well-defined critical period for stimulus-induced plasticity during development. To prevent myelin progression, we conditionally deleted Myrf, a transcription factor necessary for oligodendrocyte maturation, from oligodendrocyte precursor cells (Myrf cKO) in adolescent mice. Myrf cKO mice had significantly fewer mature oligodendrocytes and less myelin coverage in the visual cortex by five weeks of age and remained hypomyelinated well into adulthood. We examined cortical neuronal plasticity at the level of synapses by in vivo two photon microscopy and found that both spine addition and elimination rates were altered in adolescent Myrf cKOs. To determine whether these structural changes were accompanied by functional changes in neuronal plasticity, we examined ocular dominance plasticity (ODP), a form of functional plasticity in the binocular visual cortex that can be induced by monocular deprivation during the critical period (P28-35), but not in adult mice. As expected, ocular dominance was not affected by four days of monocular deprivation in adult littermate controls. By contrast, the same period of deprivation was sufficient to induce ODP in adult Myrf cKO mice. These results support the concept of myelin acting as a brake on neuronal plasticity during development and suggest that oligodendroglia and myelination play a role in shaping the maturation and stabilization of cortical circuits.

T09-027B

Astrocytes shape functional visual maps in the superior colliculus

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Astrocytes dynamically interact with neurons and contribute to cognitive functions. Yet, the role of astrocytes in sensory processing remains largely unknown. Here, we show that astrocytes are implicated in visual processing by the superior colliculus (SC), a sensory-motor structure implicated in directing behaviour. Firstly, we analysed astrocytes morphology and their anatomical organization, and reveal that astrocytes display increased complexity within the visual layer of the SC compared to hippocampus and visual cortex. We also report that astroglial networks in the SC are unusually extensive, which could be explained by the elevated expression levels of connexins, the proteins forming gap junction channels. To investigate the functional role of astrocytes in SC visual processing, we used intrinsic optical imaging and mapped the topological organization of visual responses in vivo. First, we confirmed the existence of highly robust visual functional maps related to orientation and position in visual space (retinotopy) in wild type mice. Then, we report that astroglial networks play a major role in shaping these maps, since in a transgenic mouse line in which astrocytic networks are disrupted, the visual maps are disorganized. In all, these data provide new insights into the role of neuroglial interactions in the processing of visual information.

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T09-028B

Modulation of the presynaptic local translatome by astrocytic extracellular vesicles in Alzheimer's Disease

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In the Central Nervous System (CNS), the polarization of neurons allows them to constantly monitor their surroundings in search of agents that could compromise their viability. Consequently, these highly polarized cells present an asymmetric morphology, thus implying an asymmetric distribution of proteins (Holt *et al.*, 2013). Protein synthesis is vital to guarantee the correct neuronal function. Under physiological conditions, proteins need to be appropriately sorted to the target cellular compartment where they elicit their function (Lin *et al.*, 2020). Noteworthy,

protein synthesis is not always carried out by the classical translation pathway, in which proteins are synthesized in the rough endoplasmic reticulum and after maturation, proteins are transported to the target compartment (Albets *et al.*, 2002). Protein translation can also be executed by another way based on the delivery of the mRNA transcripts to the target site, where mRNAs will be locally translated into proteins. This process is known aslocal protein synthesis (Baleriola *et al.*, 2014; Rangaraju *et al.*, 2017).

Neuronal local translation allows for a faster reaction of neural processes in response to environmental cues and contributes to the maintenance of axonal and dendritic homeostasis (Lin *et al.*, 2020). In the Peripheral Nervous System, it has been described that extracellular vesicles (EVs) secreted by Schwann cells are capable of contributing to local protein synthesis and regenerate injured nerves (Lopez-Verrilli *et al.*, 2013). Nevertheless, it is so far unclear whether glial cells are involved in local protein synthesis in CNS neurons.

Recent evidence show that in Alzheimer´s disease (AD) pathology local protein synthesis is involved in the transmission of β-amyloid pathology from the axons to the soma. In this way, the retrograde transport of proteins synthesized in the axon in response to amyloid peptide leads to pathological transcriptional changes that contribute to neurodegeneration in AD (Baleriola *et al.*, 2014). Furthermore, previous results of our research group have found evidences supporting that EVs secreted in presence of astrocytes modifies the levels of translation in axons of the CNS *in vitro*, both under physiological and AD conditions. Based on these facts, the working hypothesis is that astrocytes contribute to presynaptic local translatome through the transfer of EVs in physiological and AD conditions.

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T09-029B

Discovering the biological basis of neuronal-activity induced myelin repair

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Enhanced neuronal activity in the healthy brain can induce oligodendrocyte differentiation and *de novo* myelination leading to behavioural changes. Accordingly, clinical studies have identified non-invasive brain stimulation as a therapeutic approach to ameliorate symptomatology in demyelinating disorders such as multiple sclerosis (MS).

However, it is unknown whether benefits of brain stimulation are due to improved myelin repair in this disease (Maas & Angulo, 2021, Front Cell Neurosci). To investigate this, our lab has recently revealed that in lysolecithin demyelinated lesions in the mouse corpus callosum, optogenetic stimulation of demyelinated axons increases the number of differentiated oligodendrocytes, enhances the number of remyelinated axons and improves action potential conduction (Ortiz et al., 2019, JCI Insight). Nevertheless, the exact manner in which neuronal activity instructs oligodendrocytes to differentiate and remyelinate these active axons remains unknown. To investigate this, we use *ex vivo* calcium imaging and *in vivo* optogenetics approaches to identify the signaling mechanisms between activated axons and oligodendrocytes in demyelinated lesions of the mouse corpus callosum.

Acknowledgement

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T09-030B

Network-Specific Astrocyte Heterogeneity in the Nucleus Accumbens

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Astrocytes have been traditionally studied as a homogeneous group, however, recent research has started to evidence their heterogeneity between different brain areas and within the same region (Khakh et al. 2020, Holt et al. 2020). Our hypothesis is that specialized astrocyte subsets are responsible for the modulation of specific neuronal circuits. We have studied in vitro the astrocytic calcium responses to glutamate in the mouse nucleus Accumbens (NAc), a significant brain region not only because of its functional relevance in the reward system but also because of its integrative nature. In the NAc converge different glutamatergic signals coming primarily from the medial prefrontal cortex (mPFC), basolateral amygdala (Amyg) and ventral hippocampus (vHip), providing us the perfect environment to study the presence of specialized astrocytic networks.

In this work, we implement a tool for astrocytic study named CaMPARIGFAP, which combines the benefits of genetically encoded calcium indicators (GECIs) with permanent, large-scale labeling in a temporally precise manner. This tool performs snapshots of activity changing its fluorescence irreversibly from green to red in presence of high calcium and upon UV light delivery, allowing the simultaneous study of large areas of tissue at a specific time point in astrocytes. Using CaMPARIGFAP we studied the specific astrocytic responses to the main NAc glutamatergic inputs. In order to stimulate pathway-specifically the different afferents (mPFC, Amyg and vHip) we used an optogenetic approach based on ChrimsonR and ChR2. Synaptic responses of medium synaptic neurons in the NAc were characterized by electrophysiological recordings. NAc astrocytic calcium responses to optostimulation were monitored in real-time using CaMPARIGFAP GECI properties and in parallel NAc astrocytic population activity was studied by analyzing post-hoc CaMPARIGFAP photoconversion labeling.

Our work reveals astrocytic functional heterogeneity in the NAc regarding glutamatergic signaling, showing pathway-specific astrocytic responses mediated by mGluR5.

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T09-031B

Restorative astrocytes with reduced LCN2 activation are protective against neurodegeneration in ischemic stroke

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Astrocyte-derived lipocalin-2 (LCN2), acting as a potent mediator of neurotoxicity as well as neuroinflammation, can contribute to neuronal death. However, the exact mechanisms in regulating astrocytic LCN2 expression and cross-talk with neurons are still unknown. Our recent study shows that targeted deletion of astrocytic Na^+/H^+ exchanger 1 (NHE1) in *Gfap-Cre$^{ERT2+/-}$;Nhe1$^{f/f}$* (*Nhe1* Astro-KO) mice led to reduction of stroke infarct volume and less neuronal loss. In this study, we further investigated the underlying neuroprotection mechanisms. Control *Gfap-CreERT2$^{-/-}$;Nhe1$^{f/f}$* (wild-type) mice displayed reduction in $NeuN^+$ neurons and expression of endoplasmic reticulum (ER) homeostasis glucose-regulated protein 78 (GRP78) in post-stroke brains ($p < 0.0001$). In contrast, *Nhe1* Astro-KO mice exhibited increased number of $NeuN^+$ cells and reduced loss of $GRP78^+$ neurons ($p < 0.05$). Bulk RNA-sequencing transcriptome analysis of isolated $ACSA\text{-}2^+$ reactive astrocytes revealed upregulation of lipocalin-2 (*lcn2*) gene more in wild-type than *Nhe1* Astro-KO astrocytes. Wild-type brains displayed increased LCN2 protein expression in $GFAP^+$ astrocytes in the peri-lesion areas where $NeuN^+$ or $GRP78^+$ neurons were degenerated. But the *Nhe1* Astro-KO brains exhibited less LCN2 expressing in $GFAP^+$ astrocytes and associated with less $NeuN^+$ neuronal loss. A strong correlation between increased astrocytic LCN2 expression and loss of $NeuN^+$ or $GRP78^+$ neurons was detected. Additionally, wild-type ischemic brains showed a significant increase in the expression of ER stress marker proteins ATF4 and CHOP ($p < 0.01$) and no such elevations were detected in *Nhe1* Astro-KO ischemic brains. In summary, our study suggests that reactive astrocytes with LCN2 signaling activation play a role in neurodegeneration. Targeting *Nhe1* in astrocytes reduces LCN2 expression and promotes neuron survival after ischemic stroke.

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T09-032B

Generation of a Glia-Neuron Co-culture System Derived From Human Pluripotent Stem Cells

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Glial cells, such as astrocytes and microglia, play key roles in neurodevelopment and neurodegeneration. Studies using human pluripotent stem cell (hPSC)-derived neurons in the absence of glia do not fully represent physiological processes within the mammalian brain, or allow investigation into the complex glia-neuron interactions in disease modeling. Therefore, we developed STEMdiff™ Astrocyte kits to efficiently generate functional hPSC-derived astrocytes for in vitro modeling. hPSC-derived neural progenitor cells (NPCs) generated using the STEMdiff™ Neural Induction kit, were plated into STEMdiff™ Astrocyte Differentiation Medium, with passaging every week using the recommended protocol. On the third passage, media was replaced with STEMdiff™ Astrocyte Maturation Medium and similarly maintained for an additional two weeks. Then cell identity was confirmed using immunocytochemistry for S100β, GFAP and DCX. To assess function, we treated astrocytes with ATP and used Fluo-4AM to perform calcium imaging assay on live cells in the BrainPhys™ Imaging Optimized Medium. In separate experiments, we co-cultured the derived astrocytes with either neurons generated using the STEMdiff™ Forebrain Neuron kits alone or together with microglia generated using the STEMdiff™ Microglia kits for one week, and calculated neurite length via immunocytochemistry at the end of the cultures. Our data showed that using STEMdiff™ Astrocyte kits can result in a highly pure population of astrocytes (S100β: 72.0%±20.4%; GFAP: 33.8%±30.0%; DCX: 0.8%±1.5%; n=18). Meanwhile, calcium imaging showed that the fluorescence of the calcium signal in these astrocytes increased 8.1 times after 3 μM ATP treatment (n=11). This increase was significantly higher than control (astrocytes without ATP treatment, n=7; P=0.02) and ATP-treated NPCs (n=4; P=0.04). Interestingly, after being cultured together for one week, the number of MAP2-positive neurons was 2.2 times higher in the astrocyte co-culture group than neuron-only control (control, n=4; co-culture, n=5; P=not significant), which indicates co-culture with these astrocytes could lead to a better survival and maturation of neurons in vitro. We also analyzed the neurite length and found a 26% increase in the neuron-astrocyte co-culture group (control, n=4; co-culture, n=5; P=0.04). Finally, our preliminary data showed we can add hPSC-derived microglia generated using STEMdiff Microglia kits into the astrocyte-neuron co-culture and continue the tri-culture for at least one week. These data show that STEMdiff™ astrocyte system can generate functional hPSC-derived astrocytes which, used alongside our STEMdiff™ Forebrain Neuron and Microglia kits, enables various co-culture models for in vitro studies of neurodevelopment and neurodegeneration.

T09-033B

The dualistic role of PKCε activation in Schwann cell and peripheral sensory neurons is mediated by the neuroactive steroid ALLO.

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Protein kinase C family activity has been formerly correlated to neuropathic pain pathogenesis in peripheral sensory neurons. Lately, other studies demonstrated that the ε form (PKCε) of this superfamily plays a specific role in this process; PKCε has been strongly correlated to inflammatory pain priming and onset. Even if the role of PKCε in peripheral sensory neurons has long been studied, its pathophysiological role in Schwann cells (SCs) is still uncertain. Herein, biomolecular and pharmacological studies on rat primary SCs and dorsal root ganglia (DRG) neuronal cultures aimed at characterizing PKCε activity in both cellular types of the peripheral nervous system (PNS), and likewise to investigate the possible endogenous modulators. We investigated the hypothesis that the neuroactive steroid allopregnanolone (ALLO), renowned to be involved in analgesia, might modulates PKCε both in DRG neurons and SCs, activating distinct pathways and modulating peripheral sensitivity. Interestingly, we found that ALLO induced a dualistic PKCε action in SCs and DRG neurons, respectively. PKCε is directly downregulated in SCs and contemporarily, a PKCε paracrine upregulation occurs in DRG neurons. Interestingly, these effects imply two different cellular and molecular pathways. ALLO directly activates the classical progesterone receptor (PR) and its membrane isoform (mPR) on SCs, downregulating PKCε expression, and concurrently ALLO induces the PKCε upregulation and phosphorylation (via BDNF release and TrkB activation) in DRG neurons. Meanwhile, PKCε sensitizes the thermal and mechanical response of DRG sensory neurons via an increase in their excitability. We reported that PKCε also modulated peripheral SCs, decreasing proliferation, inducing migration and regulating gene expression.
Although the functional implications of this modulation of PKCε need further investigations, we report a novel mechanism of SCs-DRG cross-talk, highlighting a key role of ALLO in the intracellular cascade downstream inflammation. These findings are relevant in the understanding of onset and chronification of inflammatory pain of the PNS, evidencing novel and promising therapeutic targets.

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T09-034B

Astrocyte-derived extracellular vesicles modulate local translation in neurons

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Local protein synthesis is a conserved mechanism in eukaryotes by which mRNAs are localized to the cell periphery and proteins are synthesized at target sites. Local translation is especially relevant in highly polarized cells like **neurons** so that neurites can rapidly react to changes in their environment. While most of the work on localized translation in adult neurons has been done in physiological conditions, much less is known on this phenomenon in the diseased brain. The exposure of isolated axons to **β-amyloid$_{1-42}$ oligomers** (Aβo), central to

Alzheimer's disease, induces local protein synthesis and mediates neurodegeneration contributing to the disease. However, axons are not isolated in the nervous system but surrounded by other neuronal compartments or non-neuronal cells, including astrocytes, microglia and oligodendrocytes. Our laboratory is interested in **the contribution of glial cells to local translation in neurons**. Others have reported that extracellular vesicles (EVs) secreted by astrocytes are involved in the regulation of different neuronal functions. Based on these data, we hypothesize that **astrocytes release EVs that are delivered to neurons to modulate local protein synthesis in physiological and Aβo-induced conditions.**

To assess the relevance of astrocytes in neurons local proteome, we isolated somata and neurites from primary cortico-hippocampal neurons cultured in Boyden chambers in absence/presence of astrocytes and analysed the extracted proteins by LC-MS/MS. Results show that the presence of astrocytes in control conditions changes the neuritic proteome. Gene Ontology analyses show that proteins significantly regulated in the presence of astrocytes vs their absence are mainly involved in RNA binding, processing and translation. In Aβo-induced conditions, astrocytes also change neuritic proteins compared to only-neuron cultures, with translation-involved proteins among them. To determine whether these proteins are locally synthesized in neurites, we have selected 176 and are analysing their corresponding transcripts in somata and neurites.

We have also assessed if EVs are directly involved in translation regulation. Conditioned medium with or without EVs from neuron or neuron-astrocyte cultures were used to treat neurons. Removing EVs in conditioned medium from neuron-astrocyte cultures significantly changes protein synthesis in neurites. Furthermore, the treatment with the isolated EVs from neuron-astrocyte cultures recovers translation levels, suggesting that EVs are relevant for local protein synthesis in neurons. We are deeply studying EVs by LC-MS/MS to search for translation regulators. Altogether, our data provide a **new mechanism of local translation regulation** in which astrocyte-derived EVs could play an important role.

T09-035B

Role of astrocytic Ca^{2+} signaling in synaptic transmission, plasticity and memory: implications for neurodegenerative diseases

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Astrocytes are the most abundant glial cells in the central nervous system and are in close contact with synapses. In response to neurotransmitters, they increase their intracellular Ca^{2+} levels, leading to the release of neuroactive molecules. In addition, astrocytic Ca^{2+} signaling is respectively increased and decreased in Alzheimer's and Huntington's neurodegenerative diseases. Patients' memory is also altered in both diseases.

Our main hypothesis is that chronic modulation of astrocytic Ca^{2+} signaling leads to altered release of glutamate or pro-inflammatory factors from astrocytes, and consequent deficits in synaptic transmission, long-term potentiation, and memory formation.

We have tested this hypothesis *in vivo* using the mouse primary visual cortex (V1) as a model system,combined with electrophysiology, chemogenetics, genetic tools, immunohistochemistry, and behavior.

We found that chronically increasing or inhibiting astrocytic Ca^{2+} leads respectively to a depression or potentiation of visual recognition memory formation. The mechanisms underlying such changes are currently under investigation.

Our study could have profound implications in the clinic, as it may lead to a better understanding of the astrocyte role in memory deficits and in the etiology of neurodegenerative diseases.

T09-036B

The primary cilium as an organelle for astrocyte-neuron communication.

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Primary cilia are microtubule based organelles present in the plasma membrane of most cell types, including mature astrocytes and neurons. The primary cilium has emerged as a major signaling hub in the cell, however little is known about the role of this organelle in the mature brain. Data from our lab show that neuronal cilia is required for soluble amyloid beta oligomer signaling and modulation of autophagy, and that these events are modulated by physiological aging.
Here, we hypothesize that similarly to neuronal cilia, astrocytic primary cilium senses and transduces extracellular signals and that it reacts to changes in neuronal cilium. We also hypothesize that aging might alter cilia-related events in old astrocytes. To test our hypothesis, we have studied how the loss of primary cilia in neurons induces changes in astrocytes, astrocytic primary cilia, and cilia-related autophagy. For that, we have studied astrocyte reactivity and morphology in young and old IFT88::SLICK-H mice, a mouse model where cilia is lost in mature Thy1+ neurons. In these mice, we have also characterize astrocyte cilia presence and their morphology, as well as changes in the major autophagy markers. Moreover, we have deleted primary cilia in human astrocytes as well as in human neurons, and established an in vitro model of astrocyte-neuron co-culture, with the aim to study the dynamics of astrocyte and neuronal cilia changes between these two cell types. Overall, we aim to understand the role of mature astrocytic primary cilia in the brain, as well as its interplay with neurons and their possible changes during aging.

T09-037C

Satellite glia cell-proprioceptor interactions in dorsal root ganglia

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Proprioception is a sensory modality that informs the central nervous system about the precise body and limb

position in order to estimate the state of movements. Thus, proprioceptive neurons play a major role in locomotion and posture. Their cell bodies are located in the dorsal root ganglia (DRG) and are tightly enwrapped by glial cells, called satellite glial cells (SGCs). SGCs can sense neuronal activity through their Gq protein coupled receptors (Gq GPCRs) located at their plasma membrane. Under physiological conditions, SGCs maintain neuronal extracellular homeostasis *via* their hemichannels, channels or transporters. Our laboratory has recently found that *ex vivo* activation of Gq GPCR Ca^{2+} signaling in SGCs leads to activation of proprioceptors *via* a purinergic system.

To examine further whether such SGC-induced Ca^{2+} elevations in proprioceptors could impact proprioceptive function in a more physiologically-relevant *in vivo* context, we used a transgenic mouse line expressing a DREADD receptor under the control of the GFAP promoter. We observed several abnormal limb positioning in DREADD mice as compared to controls, suggesting that SGC Gq GPCR Ca^{2+} signaling alters sensorimotor function *in vivo*. However, a contribution of astrocytes on spinal interneuron and/or motor neuron functions or on brain cannot be excluded.

To overcome this technical limitation, we have developed a different approach based on Adeno-Associated Viral (AAV) delivery of transgene selectively in SGCs of single DRG *in vivo*. This approach will allow us to avoid possible confounding results due to DREADD expression in glial cells elswhere than in DRG.Our results have implications in diseases with DRG SGC impairments and sensorimotor disorders.

T09-038C

Astroglial gap junctions strengthen hippocampal network activity by sustaining afterhyperpolarization

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Astroglial networks formed by gap-junction channels promote population activity by modulating neuronal excitability and network synchronization. However, the underlying cellular and molecular mechanisms remain unclear. Numerous alterations of neuronal membrane and synaptic properties were identified in astroglial connexin-deficient mice, questioning their relative contribution to the altered bursting pattern. Here mice with disconnected astrocytes, we found that astroglial networks regulate neuronal bursting patterns via dynamic regulation of extracellular potassium levels. We then examined the physiological and molecular targets underlying the astroglial-mediated potassium regulation of neuronal patterns by an interdisciplinary approach combining electrophysiology and modeling. We identified neuronal afterhyperpolarization as the key parameter underlying bursting patterns regulation by extracellular potassium in mice with disconnected astrocytes. We confirmed experimentally this prediction and revealed that astroglial network-control of extracellular potassium sustains neuronal afterhyperpolarization. Altogether, these data identify the mechanism by which astroglial gap-junctions strengthen neuronal population bursts, thus pointing to selective astroglial gap-junction modulators as alternative therapeutic targets to control aberrant activity in neurological diseases.

T09-039C

Altered perisynaptic astrocyte processes in a mouse model for the leukodystrophy MLC

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Loss of function of the astrocytic membrane protein MLC1 is the primary genetic cause of the rare white matter disease Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC). MLC is characterized by chronic edema of the brain white matter, myelin vacuolization, astrocyte swelling and disrupted brain ion and water homeostasis. MLC1 is prominently present around fluid barriers in the brain, such as in astrocyte endfeet contacting blood vessels and in processes contacting the meninges.

Here we show that MLC1 protein is also found in so-called perisynaptic astrocyte processes (PAPs) in the CA1 region of the hippocampus. PAPs are specialized astrocyte structures that closely interact with pre- and postsynaptic compartments in a structure known as the tripartite synapse. By studying PAP morphology in *Mlc1*-null mice using electron microscopy, we find that most ultrastructural properties of excitatory synapses in hippocampal CA1 are unaltered. However, the PAP protrusions directed towards excitatory synapses are shorter in *Mlc1*-null mice. PAPs contain high levels of glutamate transporters and are essential for tight control of the synaptic glutamate transient. Surprisingly, we find that basal glutamatergic synaptic transmission is mostly unaffected in *Mlc1*-null mice. In addition, these mice show normal hippocampal long-term potentiation (LTP). We are currently investigating whether the time course of the glutamate transient and/or glutamate spillover is altered in *Mlc1*-null mice by using the fluorescent glutamate sensor iGluSnFR.

To test if the altered morphology of astrocyte PAPs has behavioral consequences, we tested contextual fear conditioning in *Mlc1*-null mice. Mice were placed in a fear conditioning chamber and allowed to explore before receiving a single foot shock. Retention of the fear memory was assessed by returning mice to the conditioning box 24 hours after training and measuring freezing behavior. *Mlc1*-null mice show reduced freezing, indicating a reduced retention of the fear memory.

To conclude, our study uncovers a surprising role for the astrocytic protein MLC1 in regulating astrocyte PAP morphology at the tripartite synapse. Hippocampal basal glutamatergic synaptic transmission and long-term synaptic plasticity are intact in *Mlc1*-null mice, suggesting no effect of altered PAP protrusions on synaptic physiology. In contrast, the reduction in contextual fear memory retrieval in *Mlc1*-null mice suggests that synaptic MLC1 expression is involved in recent fear memory formation. More in-depth analysis of glutamatergic transmission is ongoing to explain this apparent discrepancy.

Acknowledgement

M.S. Kater and K.F. Baumgart contributed equally to this work

M.H.G. Verheijen and R. Min share senior authorship

T09-040C

Characterization of microglial morphodynamics during sleep-wake cycle

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Microglial cells, the resident immune cells of the brain, have particularly dynamic processes. Various studies suggested that beyond a possible role in surveillance, microglial dynamics could be linked to synaptic mechanisms and neuronal activity. However, signaling pathways modulating the neuronal control of microglial motility remain largely unknown. As vigilance states are characterized by distinct neuronal activities, we first assessed whether and how microglial morphodynamic is modulated by vigilance states. Our second aim was to identify neuronal signaling pathways involved in the regulation of microglial dynamic. We focused on two signaling pathways related to microglial chemotactism: the fractalkine pathway, a chemokine released by neurons that binds to CX3CR1 expressed by microglia, and the serotoninergic signaling, through metabotropic HTR2B receptors also expressed by microglia.

Microglial morphodynamic changes were studied through *in vivo* transcranial imaging using two-photon microscopy, while electroencephalogram and electromyogram were recorded to monitor vigilance states. Transgenic CX3CR1-GFP mice were used to observe and analyze fluorescent microglial cells. We first evaluated the impact of fractalkine receptor depletion to figure out its role in microglial dynamic. Then, we studied the impact of serotonin signaling by triggering an accumulation of serotonin in the brain. Consequently, we performed morphodynamic analysis with custom MatLab programs to evaluate process motility and cell complexity.

Preliminary results indicate a decrease in microglial morphodynamic during slow wave sleep. We also found that a depletion of the fractalkine receptor abolished these sleep-induced morphodynamic changes, indicating that fractalkine could be involved in mechanisms related to sleep. Independently of vigilance states, we found that serotonin build up increased microglial motility, with no effects on complexity, which indicate that motility and complexity of microglial cells could be regulated through different signaling pathways.In conclusion, this work identifies fractalkine and serotonin as potent modulators of microglial dynamics in physiological conditions. This study will lead to a better understanding of microglial functions in the context of synaptic transmission and plasticity.

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T09-041C

Astrocytes from distinct nigrostriatal brain regions secrete extracellular vesicles able to mediate neuroprotection in cellular models of Parkinson's disease

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Astrocytes (AS) are the main actors in the processes which regulate the homeostasis of dopaminergic (DAergic) neurons in both physiological and pathological conditions [1]. In Parkinson's disease (PD) AS may mediate either destructive or beneficial events. PD is a chronic neurodegenerative disease characterized by the progressive loss of DAergic neuronal cell bodies in the ventral Midbrain (VMB) and their terminals the Striatum (STR), with consequent Dopamine depletion. In this context, when activated by the chemokine CCL3, AS exert a robust DAergic neuroprotection both in cellular and pre-clinical models of PD [2]. However, the complex intercellular signaling between AS and neurons has not been fully elucidated, yet. One of the strategies used by cells to transfer information to adjacent or distant cells is by extracellular vesicles (EVs). EVs are nanometric structures containing cell deriving material (e.g. RNAs, proteins, lipids and others), released by almost all cells in the microenvironment. Based on their dimension, EVs are classified as small (< 200 nm) or medium/large (>200 nm) EVs [3]. Among small EVs, exosomes and microvesicles are the most investigated. We herein isolated EVs derived from VMB- and STR-AS, both in basal and CCL3-activated conditions, with the aim to evaluate their possible involvement in the AS-neuron crosstalk. EVs were purified from AS supernatants by ultracentrifugation and characterized for their dimension and concentration. We found that AS secrete vesicles with a dimension of ∼100 nm, positive for CD63, CD9 and Alix, all small EV markers. In basal conditions, VMB-AS release more EVs than STR-AS, and only VMB-AS respond to CCL3 by producing more EVs, demonstrating that different brain areas specifically affect the EV secretion rate of AS. Next, the functional effects of AS-EVs were evaluated on differentiated SH-SY5Y target cells, under neurodegenerative conditions. SH-SY5Y cells were pre-treated with AS-EVs and then exposed to H_2O_2. As a result, caspase activation was found significantly reduced with AS-EVs, but especially with CCL3 AS-EVs. Furthermore, we assayed AS-EVs in a more specific PD model, by treating SH-SY5Y cells with 1-methyl-4-phenylpyridine (MPP^+) neurotoxin which significantly reduce the mitochondrial functionality [4]. By high resolution respirometry technique, we observed a recover of mitochondrial complex I activity in the presence of all AS-EV samples. Notably, only VMB-AS-EVs fully restored ATP production in MPP^+-injured SH-SY5Y cells. For the first time, to our knowledge, our study shows the existence of specific brain region-linked mechanism(s) for AS-EV secretion, with possible functional implications in the intercellular communication within the nigrostriatal area, in the context of PD.

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T09-042C

Neuron-glia crosstalk mediate the neurotoxic effects of ketamine via extracellular vesicles

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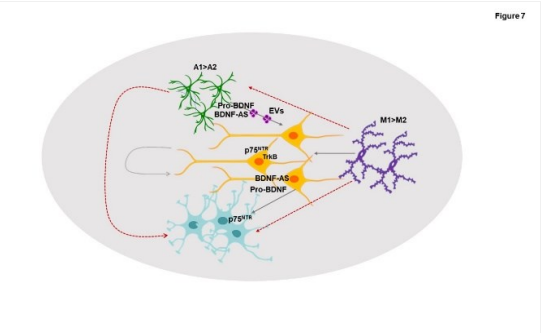
Background: General anesthetics (GA) are associated with neurodevelopmental abnormalities including cell death, cognitive and behavioral changes. There is now powerful evidence for non-cell autonomous mechanisms in almost every pathological condition in the brain, especially relevant to glial cells, mainly astrocytes and microglia, that exhibit structural and functional contacts with neurons. These interactions were recently reported to occur via the secretion of extracellular vesicles (EVs). Here, we employed primary human neural cells to analyze ketamine effects focusing on the functions of glial cells and their polarization/differentiation state. We also explored the roles of extracellular vesicles (EVs) and different components of the BDNF pathway.
Methods: Ketamine effects were analyzed on human neuronal and glial cell proliferation and apoptosis and astrocytic (A1/A2) and microglial (M1/M2) cell activation were analyzed. The impact of the neuron-glial cell interactions in the neurotoxic effects of ketamine was analyzed using transwell co-cultures. The role of the brain-derived neurotrophic factor (BDNF) pathway, was analyzed using RT-PCR, ELISA western blot and gene silencing. EVs secreted by ketamine-treated cells were isolated, characterized and analyzed for their effects in neuron-glia cell interactions. Data were analyzed using analysis of variance or a Student's t test with correction for data sets with unequal variances.
Results: Ketamine induced neuronal and oligodendrocytic cell apoptosis and promoted the expression of pro-inflammatory astrocytes (A1) and microglia (M1) phenotypes. Astrocytes and microglia enhanced the neurotoxic effects of ketamine on neuronal cells, whereas neurons increased oligodendrocyte cell death. Ketamine modulated different components in the BDNF pathway: decreasing BDNF secretion in neurons and astrocytes while increasing the expression of p75 in neurons and oligodendrocytes. In addition, ketamine treatment increased the lncRNA BDNF-AS levels and the secretion of pro-BDNF secretion. We found an important role of EVs secreted by ketamine-treated astrocytes in neuronal cell death by delivering BDNF-AS.
Conclusions: Ketamine neurotoxicity involves both autonomous and non-cell autonomous mechanisms andomponents of the BDNF pathway expressed by neurons and glial cells represent major regulators of ketamine effects. We demonstrated for the first time a role of EVs as important mediators of ketamine effects by the delivery of specific non-coding RNAs. These results may contribute to a better understanding of cellular and molecular mechanisms underlying ketamine neurotoxic effects in humans and to the development of potential approaches to

decrease its neurodevelopmental impact.



Neuron-glia interactions, the BDNF pathway and extracellular vesicles in ketamine effects
The effects of ketamine on various components of the BDNF pathway, the interactions of neuron and glial cells and the role of EVs as mediators of these interactions are depicted in this diagram.

T09-043C

Neural-glia interactions mediate the loss of perisomatic inhibitory synapses in *Toxoplasma gondii* infection

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Prolonged infection and inflammation within the brain can alter the connectivity and function of neuronal circuits. The intracellular protozoan parasite, *Toxoplasma gondii*, is one pathogen that can chronically infect the brain and lead to encephalitis and seizures. Currently, 1 in 3 humans are infected with *Toxoplasma gondii* worldwide, and these infections have been identified as a considerable risk factor for developing complex neurological and psychiatric disorders that arise from alterations in assembly and maintenance of synapses, such as schizophrenia. To directly assess changes in inhibitory synapses, we employed serial block face scanning electron microcopy and quantified perisomatic synapses in neocortex and hippocampus following parasitic infection. Ultrastructural analyses, in combination with genetic and immunohistochemical tools, revealed that persistent infection not only led to a significant loss of inhibitory perisomatic synapses, it also induced the ensheathment of neuronal somata and perisomatic nerve terminals by activated myeloid-derived cells (including microglia), suggesting they may displace or phagocytose synaptic elements in long-term infection. How might microglia contribute to inhibitory synapse loss in parasitic infection? One potential mechanism is the innate complement cascade, an immune

response by which phagocytic cells, such as microglia, clear pathogens. Importantly, we saw substantial upregulation of C1q and C3, classical complement components that have been shown to have critical roles in microglia-induced synapse loss during developmental synaptic refinement, neurodegeneration and disease. Using transgenic and knockout mouse models, our current studies explore the innate complement pathway as a mechanism by which microglia phagocytose inhibitory nerve terminals in long-term parasitic infection and highlight novel ways in which immune molecules regulate glial and neuronal interactions in parasitic brain infection.

T09-044C

Compartment-Specific Nanomodular Signaling Drives Functional Tripartite Synapse Assembly

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At tripartite synapses, astrocytes surround synaptic contacts, but how astrocytes contribute to the assembly and function of synapses remains largely unresolved. By profiling astrocyte-specific ribosome-bound mRNAs near the completion of hippocampal excitatory synapse maturation, we found that several well described synaptic cell adhesion molecules are also enriched in astrocytes (e.g. Cadm1, PTPRF, and Nrxn1). Here we focused on the role of Nrxn1 in tripartite synapse assembly owing to its ability to recognize dozens of postsynaptic ligands, its relatively high enrichment in astrocytes and significant role in neurodevelopmental and psychiatric disorders. We show that neurexin-1α, but not other Nrxn1 isoforms, is abundantly expressed by astrocytes in the forebrain. Using superresolution microscopy, we show that a majority of hippocampal excitatory synapses contain both presynaptic and perisynaptic astrocytic Nrxn1 nanoclusters. Supporting a mechanism for postsynaptic neurons to discriminate between astrocytic and neuronal Nrxn1 nanoclusters, we observed that astrocytic neurexin-1α, but not neuronal neurexin-1α, is highly modified by heparan sulfate. Moreover, astrocytic neurexin-1α is uniquely alternatively spliced and invariably contains an insert in splice-site #4, thereby restricting the range of ligands to which it binds. Deletion of neurexin-1 from astrocytes or neurons does not alter synapse numbers or synapse ultrastructure, but differentially impairs synapse function. At hippocampal Schaffer-collateral synapses, neuronal neurexin-1 is essential for normal NMDA-receptor-mediated synaptic responses, whereas astrocytic neurexin-1 is required for silent synapse maturation and for long-term potentiation. Thus, we identify a molecular logic for compartment-specific instruction of functional assembly of tripartite synapses and unveil an ontogenetic window in which astrocytes instruct synapse maturation independently of synaptogenesis.

T09-045C

Small extracellular vesicles from primary astrocytes exert a neuroprotective effect on damaged primary neuronal cultures.

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In recent years, the importance of the small extracellular vesicles from astrocytes on neurological diseases has been widely demonstrated. A small extracellular vesicle is a small lipid bilayer particle (less than 150 nm) released from cellular multivesicular bodies. These extracellular vesicles can contain multiple different types of molecules and organelles. Although the physiological effects of the small extracellular vesicles are not fully understood, they are related with the type and health status of the cell that originates them[1]. The small extracellular vesicles from astrocytes could have neurogenic, synaptogenic, and neuroprotective effects.[2]

For that reason, we aim to study the effect of small extracellular vesicles from early passage rat primary astrocytes cultures on mouse primary neuron cultures damaged or not with epoxomicin. The treatment with epoxomicin generates a model of disrupted proteostasis, which is also present in aging and some neurodegenerative diseases.

Our results show a significant neuroprotective effect of the treatment with these small extracellular vesicles on the damaged neuron cultures with epoxomicin. As for the possible mechanism by which these small extracellular vesicles make their effect, we study different cellular processes altered in our neuronal model. For example, we corroborate that the treatment with small extracellular vesicles shows a significantly improved mitochondrial function on neurons treated with epoxomicin. We also observe how the treatment with small extracellular vesicles significantly restores the decreased GSH levels present on neurons treated with epoxomicin.

Thus, the astrocyte extracellular vesicles have a neuroprotective effect on neurons by improving the mitochondrial function and the antioxidant response. It is necessary to study further consequences from the treatment with astrocyte derived small extracellular vesicles on damaged neurons and on animal models of neurodegeneration. It would be possible to use them as a treatment against different neurological disorders and as carriers of several neuroprotective compounds.

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T09-046C

Investigating the structure and function of synaptic contacts between neurons and oligodendrocyte precursor cells in vivo

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Oligodendrocyte precursor cells (OPCs) comprise a heterogeneous cell population that inhabits a continuum of different stem-like abilities: they can be quiescent, proliferate to give rise to more OPCs, or differentiate into myelinating oligodendrocytes. Heightened activity levels in neighbouring neuron populations can increase both OPC proliferation rates and myelin production by oligodendrocytes. To integrate neuron activity levels, OPCs use both synaptic and non-synaptic mechanisms, with the latter playing a key role in instructing ongoing myelination efforts. However, despite knowing for over 20 years now that neurons make glutamatergic and GABAergic synaptic contacts with OPCs, the functional relevance of these connections is still unknown.

Here, we address this question using zebrafish as an animal model to (1) study the pattern of synaptic connections between neurons and OPCs in vivo and to (2) probe their role in neural circuit function. We are able to label OPC synapses using a combination of transgenic lines such as olig1:KalTA4 and newly generated synaptic-marker probes UAS:psd95-fingR-XFP or UAS:gephryn-fingR-XFP, which are fluorescently tagged intrabodies that specifically label endogenous glutamatergic or GABAergic post-synaptic sites, respectively. We have identified that only sub-populations of OPCs have gephryn-positive inhibitory synapses, highlighting a key difference to resident neurons in which every cell tested displayed clear synaptic puncta present alongside neurites and somatic regions. We will present data showing the diversity of OPC synaptic contacts relative to neurotransmitter type, and OPC properties, as well as the fates of these contacts as OPCs progress along their lineage.

To investigate the function of neuron-OPC synapses we are using a combination of optogenetic stimulation of either glutamatergic or GABAergic neurons together with electrophysiological recordings of different OPC subgroups *in vivo*. We will present data where we directly probe the presence and strength of connections between different neuron populations and OPCs, and correlate this with anatomical information about their relative synaptic architectures, and fates in vivo.

Together, our work will reveal the heterogeneity of neural activity integration by OPCs, and allow us to test for the first time whether neuron-OPC synapses are an integral part of the canonical OPC myelinogenesis pathway, or whether they play a role in yet undescribed aspects of neural circuit function and development.

T09-047C

Neuropathic pain: Characterisation of adrenoceptors analgesic downstream signalling in Spinal Glial cells

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Neuro-glial interaction in the spinal cord is important for neuropathic pain development after peripheral nerve injury. Injured neurons release many molecules, among those ATP and chemokines, which lead to the activation of microglia. Activated microglia show increased production of neuroactive and pro-inflammatory factors and the entrance of calcium through the $P2X_4$ channels augments the phosphorylation (i.e. activation) of the mitogen-activated protein kinases, such as p38, ERK and JNK, which participate in central sensitization, astrocytic activation and generation of pain hypersensitivity. Several preclinical studies using neuropathic pain models have demonstrated that the descending noradrenergic pathway is crucial to reduce pain at the spinal cord level. Indeed, the application of specific adrenoceptor (AR) agonists such as Clonidine, a specific α2-AR agonist, or Formoterol, a

β2-AR agonist, produces an analgesic effect in patients and in mouse models, respectively.
The anti-nociceptive effects of noradrenaline in the spinal dorsal horn implicate multiple mechanisms involving neurons, glial cells and their crosstalk. However, the precise role of glial adrenergic receptors in neuropathic pain remains elusive, especially how activation of their downstream signalling in microglial cells influences neuronal activity in the spinal cord.
We demonstrate that in spared nerve injury (SNI) operated mice of both genders, intraperitoneal administration of Clonidine three days after SNI surgery is able to significantly revert the increased density and activation of microglia in the ipsilateral spinal dorsal horn, compared to the control saline-injected mice. Moreover, we show that six days after the SNI operation, a single injection of Formoterol relieves the mechanical and thermal hypersensitivity in neuropathic-model mice, while a second injection after three weeks reduces only the mechanical hypersensitivity. The immunofluorescence analysis on Formoterol-treated mice suggests that the main player in neuropathic pain development is microglia while astrocytes are more important for the maintenance of neuropathic pain.
In conclusion, our study supports the analgesic effects of adrenoceptors activation in neuropathic pain conditions and suggests a mechanism involving the silencing of spinal microglia.

T09-048C

Investigating gene expression changes in oligodendrocyte precursor cells in response to neural activity

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Oligodendrocyte precursor cells (OPCs) integrate nervous system activity via expression of a variety of neurotransmitter receptors, which positively regulates myelination in the central nervous system. However, a large population of OPCs does not directly differentiate in response to neural activity, suggesting additional roles of this cell population. Therefore, we aim to investigate how neural activity affects OPCs in other ways besides their changes in cell fates.
We show that systemic application of the voltage gated potassium channel blocker 4-Aminopyridine [4-AP] stimulates neural activity in zebrafish larvae. Chronic, low-dose treatment with 4-AP enhanced spontaneous swimming behaviour and neuronal activity as assessed by in vivo imaging of the genetically encoded calcium sensor GCaMP6s. Similarly, calcium signalling activity was also increased in OPCs as revealed by GCaMP imaging. 4-AP-induced effects lasted overnight, and did not cause inflammation (defined by macrophage recruitment) and signs of tissue damage after treatment from 3 dpf to 4 dpf.
In order to identify gene expression changes in OPCs in response to enhanced neural activity, we performed fluorescence-activated cell sorting of OPCs from transgenic Tg(olig1:memYFP) zebrafish that have been exposed to 4-AP overnight. RNA sequencing of these cells was performed.
Gene ontology analysis of differentially regulated genes revealed categories relating to intracellular signalling (protein dephosphorylation, responses to extracellular stimuli), glycoprotein metabolism, protein catabolism and others. Interestingly, one of the biggest group of differentially regulated genes are associated with axon guidance

and cell avoidance. We will present data confirming expression of some of these candidates specifically in OPCs using *in situ* hybridisation and immunofluorescent staining, and we will present phenotypes that animals exhibit when some of these genes have been knocked out using CRISPR/Cas9.
Together, the work presented will define potential mechanisms by how neural activity affects OPCs and how this might in turn alter neuronal function; potentially defining new roles for OPCs beyond myelination.

T09-049C

OPC shapes medial prefrontal cortical inhibition by regulating interneuron apoptosis and myelination via $GABA_B$ receptor

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Myelination of cortical inhibitory neurons is essential for fast and timed conduction of action potentials, thereby ensuring fine-tuning of cortical circuit activity. GABAergic signaling from interneurons to oligodendrocyte precursor cells (OPCs) controls their differentiation into oligodendrocytes, and thereby determines interneuron myelination.
To investigate the role of GABAB receptors of OPCs in this process, we crossbred NG2-CreERT2 knock-in mice with GABAB1R floxed mice to conditionally knockout (cKO) GABAB1 receptors specifically from OPCs and their progeny. We induced cKO at postnatal day 7 and 8, and analyzed the region of medial prefrontal cortex (mPFC) at the age of 9 weeks. In the mPFC of cKO mice, we observed a reduced OPC differentiation, decreased MBP expression and changes of the paranodal loops. By performing double immunostaining of parvalbumin (PV) and MBP, we found that hypomyelination mainly occurred for the PV+ interneurons. Furthermore, we observed a reduced levels of the vesicular GABA transporter (vGAT) in the postnatal mice by immunohistochemistry and attenuated spontaneous inhibitory postsynaptic currents (sIPSCs) in young adult mice by electrophysiology. Consequently, GABAB receptor-deficient OPCs received significantly less GABAergic input and their differentiation was suppressed. In addition, we found an overpopulation of PV+ neurons in the mutant mPFC of adult mice. By analyzing the programmed cell death of interneurons during development, we found that OPCs release TWEAK (Apo3l), a weak apoptotic factor of the TNF super family. During early stages of normal CNS development, TWEAK contributes to the removal of supernumerous interneurons. We conclude from our data that interneuron-OPC signaling via GABAB receptors is essential for the regulation of TWEAK. A subsequent behavioral analysis revealed a severe impairment of social cognitive behavior in the cKO mice.
In summary, our findings uncover a bidirectional communication pathway between interneuron and OPC. During development, OPCs sense GABA via GABABR and release TWEAK to optimize interneuron population and function, which is pivotal for interneuron myelination and network function in the medial prefrontal cortex.

T09-050C

SHH-signaling as an intermediary between retinal inputs and astrocytes for the recruitment of GABAergic interneurons

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Visual thalamus receives direct inputs from retinal ganglion cell (RGC) axons and is important for both image-forming and nonimage-forming visual functions. Recent studies have shown that these RGC inputs play important roles in the development of cell types and circuits in these regions. For example, retinal inputs regulate the long-distance recruitment of GABAergic interneurons into two regions of visual thalamus – the dorsal and ventral lateral geniculate nuclei. Our lab recently discovered that these retinal inputs induce astrocytes to generate fibroblast growth factor 15 (FGF15), a potent motogen that is essential for interneuron migration into these regions. However, how retinal inputs induce the astrocytic expression of FGF15 remains unresolved. Here, we tested the role of RGC-derived Sonic Hedgehog (SHH) in this process since RGCs are known to release SHH in the brain and SHH is known to induce FGF15 expression in other parts of the embryonic brain. Using transcriptomic analysis, *in situ* hybridization, and reporter line, we observed that thalamus-projecting RGCs express SHH, and SHH receptors and signaling pathway components are expressed in the developing visual thalamus. Specifically, we discovered thalamic astrocytes express Patched-1 (PTCH1), Smoothened (SMO), and glioma-associated oncogene (GLI) transcription factors. Our data revealed a significant decrease in the thalamic expression of *Fgf15* mRNA in the mice lacking SHH specifically from RGCs, suggesting the importance of this axo-glial signaling pathway in the development of visual thalamus. Taken together, our results shed light on novel ways in which astrocytes mediate the recruitment of interneurons into neonatal visual thalamus

T09-051C

Cuprizone-demyelination alters the number and location of neurons that form synaptic connections with oligodendrocyte progenitor cells

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Oligodendrocyte progenitor cells (OPCs) receive direct synaptic input from neurons in the developing and mature brain, however, the number of synaptic connections has been reported to change during demyelination and remyelination. To determine how the number and location of neurons that form synaptic connections with OPCs, changes with cuprizone-induced demyelination or during remyelination, we studied the synaptically-connected cells using a combined viral labelling and electrophysiology approach. We rendered OPCs susceptible to infection by a

monosynaptic rabies virus (SADΔG-GFP-EnvA), by delivering tamoxifen to young adult *Pdgfrα-CreER^{T2} :: Rosa26-gp4-TVA* mice. Demyelination was then induced by 5-weeks of cuprizone feeding (0.2% w/w). 24h after mice were returned to a normal diet, we injected the rabies virus into the corpus callosum, underlying the retrosplenial and parietal cortices (bregma -1.5mm). A small number (~1-20) of "starter" OPCs were GFP-labelled at 3 days, before the virus moved retrogradely to label synaptically connected neurons (~400-1900). In the healthy brain, callosal OPCs received synaptic input from cortical and subcortical neurons, with the majority (~84%) originating from the ipsilateral hemisphere. Callosal OPCs also received synaptic input from neurons in CA1 of the hippocampus (~59% of GFP$^+$ neurons). Following demyelination, the total number of GFP$^+$ neurons was reduced by ~80%, and the location of the connected neurons was also altered, with callosal OPCs receiving ~8% fewer synaptic inputs from neurons in the contralateral cortex, and ~45% fewer inputs from hippocampal neurons. These data suggest that the early stages of demyelination are associated with less synaptic communication between OPCs and neurons, and that connected OPCs may prioritise communication with the neurons that are most affected by demyelination.

T09-052C

Glial Synaptobrevin mediates peripheral nerve insulation, neural metabolic supply and is required for motor function

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Peripheral nerves contain sensory and motor neuron axons coated by glia cells whose interplay ensures function, but molecular details are lacking. SNARE-proteins typically mediate the exchange and secretion of cargo by enabling the fusion of vesicles with target organelles, but how glial SNAREs contribute to peripheral nerve function is unknown. We here identify non-neuronal Synaptobrevin (Syb) as the essential vesicular SNARE in *Drosophila* peripheral glia to insulate and metabolically supply neurons. We show that glial expression of a potent inhibitor of SNARE-mediated fusion reactions, Tetanus neurotoxin light chain (TeNT-LC), causes nerve disintegration, defective axonal transport, tetanic muscle hyperactivity, impaired locomotion and lethal paralysis. While TeNT-LC blocks neurotransmitter exocytosis in neurons by cleaving neuronal Synaptobrevin (n-Syb) it targets non-neuronal Synaptobrevin (Syb) in glia which it cleaves at low rates: Glial Syb knockdown (but not that of n-Syb) phenocopied glial TeNT-LC expression whose effects were reverted by expression of a TeNT-LC-insensitive Syb mutant. We link Syb-necessity to two distinct glial subtypes: Impairing Syb function in subperineurial glia disrupted nerve morphology, axonal transport and locomotion, likely because nerve-isolating septate junctions could not form due to the mistargeting of their components (like the cell adhesion protein Neurexin-IV). In axon-encircling wrapping glia, Syb knockdown left nerve morphology and locomotion intact but impaired axonal transport, likely by the mistargeting of metabolite shuffling monocarboxylate transporters and a thus disrupted neural metabolic supply. Our study identifies crucial roles of Syb in various glial subtypes to ensure their structural and functional interplay needed for proper nerve function, animal motility and survival.

T09-053C

Lactate metabolism in the control of microglial function

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Microglia are the tissue-resident macrophages of the brain. Beyond their innate immunity roles, they are implicated in a variety of physiological processes required for proper brain development, including removal of apoptotic neurons and remodeling of synapses. Not surprisingly, dysregulation of microglial function is linked with the onset of neuropathology. Accumulating evidence points towards the involvement of metabolism and differential substrates catabolism in the regulation of immune cells, including microglia. In particular, lactate, which sustains brain energetics and increases in response to neuronal activity, was shown to regulate inflammatory responses in peripheral immune cells. However, the physiological role for lactate in modulating microglial function is still unexplored. In order to address this question, we generated a microglia-specific conditional *knock out* (cKO) mouse model for the monocarboxylate transporter 4 (MCT4), which we describe to be specifically upregulated in microglia upon lactate exposure and which is known to be implicated in lactate transport. We analyzed key microglia features during postnatal development, and we found alterations in microglial density and in CD68+ endosomal/lysosomal structures in the hippocampus of two-week-old cKO mice. This was associated with alterations in the protein levels of presynaptic markers and changes in excitatory post-synaptic currents, indicating that microglia-specific depletion of MCT4 is sufficient to considerably affect neuronal development and function. Additionally, adult cKO mice present an anxiety-like phenotype. In summary, this study highlights the importance of microglial metabolism for correct brain maturation, emphasizing how metabolic flexibility, and in particular lactate metabolism, could be functionally coupled to microglial regulation. Given the established role of microglia in neuropathology, a better mechanistic understanding of lactate-dependent modulations may be relevant for targeting microglia in brain disease.

T09-054D

Parkinson's disease-associated LRRK2-G2019S mutation disrupts glia-neuron crosstalk and impairs dopaminergic neurodevelopment

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Accumulating evidence indicates that a complex interplay between genes and environmental factors contribute to dopaminergic (DA) neuron demise in Parkinson's disease (PD). Among PD-associated genes, mutations in the Leucine-Rich Repeat Kinase (LRRK2) gene are the most common cause of familial late onset PD. The low penetrance of LRRK2 mutations, the low disease concordance in relatives, and the lack of substantial neurodegeneration in the LRRK2 mouse models would all suggest that other genetic or environmental hits are required for the degeneration of DA neurons. Here, we hypothesized that aging and LRRK2-mediated immune dysregulation contribute to the derangement of the neuron-glia crosstalk.

To investigate the impact of LRRK2 on neuron-glia interactions, primary ventral midbrain (VM) glial-neuron cultures from wild type (Wt) and transgenic-(Tg)-LRRK2-G2019S mice were allowed to grow for different time-intervals (T=0-18 days in vitro, DIV) and fluorescence immunocytochemistry coupled with confocal laser scanning microscopy using neuronal and glial cell markers, were applied to monitor astrocyte, microglia, and mesencephalic neuron development, in either purified or mixed glia-neuron cultures. To recapitulate the interaction between G2019S with inflammatory and/or neurotoxic insults, the primary VM cultures were primed with different inflammogens and/or DA neurotoxins mimicking PD-mediated neuronal death.

LRRK2 G2019S markedly affected glial morphology, neurotrophic and prosurvival properties, as reflected by the poor neuronal development and delayed acquisition of the mature DA phenotype in mixed cultures. G2019S astrocytes exhibited greater sensitivity to MPP^+ and LPS challenge, enhanced production of reactive oxygen/nitrogen species, and deficient mitochondrial respiration, as compared to Wt counterparts. G2019S microglia further exacerbated astroglia activation and inhibited its neuroprotective function in mixed cultures, compared to Wt controls.

These results suggest that the LRRK2-G2019S mutation disrupts glial development and impairs glia-neuron crosstalk resulting in increased vulnerability and loss of mesencephalic neurons. Current work is addressing the biochemical and molecular mechanisms underlying such LRRK2-dependent glial-neuron crosstalk. (ER-2020-23669429 – Eranet-CoEN2017 Pathfinder).

T09-055D

Aging induces morphofunctional remodeling of astrocytes and reduces LTP in the mouse hippocampus

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Brain aging has been commonly studied from a neuronal perspective. However, brain function depends on a complex interplay of different cellular and subcellular elements including astrocytes. Like neurons astrocyte morphology and function are affected by aging. However, how morphological and functional changes are linked to each other and synaptic plasticity is still poorly understood. We loaded astrocytes with Alexa Fluo 594 through a patch pipette. Cell morphology and dye-diffusion to the neighboring cells were revealed with two-photon imaging. Astrocyte morphology was analyzed with Sholl analysis and volume fraction (VF) analysis. We observed decreased number of astrocytic branches and reduced VF occupied by leaflets in the aged brain. Astrocytic domain shrunk

leading to reduced astrocyte-to-astrocyte coupling. Astrocytic dystrophy may be associated with reduced synaptic coverage, which in turn would promote spillover of synaptically released glutamate and K^+. To test this possibility, we recorded synaptically induced glutamate transporter-mediated current and K^+ current in voltage-clamped astrocytes. We observed larger activity-dependent prolongation of both currents, which is consistent with less efficient glutamate and K^+ clearance by astrocytes. Enhanced glutamate spillover facilitates activation of extrasynaptic NMDA receptors, which in turn lead to smaller LTP magnitude. Indeed, we observed that LTP in aged mice was smaller and was less sensitive to partial blockade of glutamate transporters with TBOA than in adult mice. We concluded that age-dependent astrocyte dystrophy might be responsible for reduced synaptic plasticity and the associated decline in learning and memory.

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T09-056D

Schwann cell plasticity regulates neuroblastic tumor cell differentiation via epidermal growth factor-like protein 8

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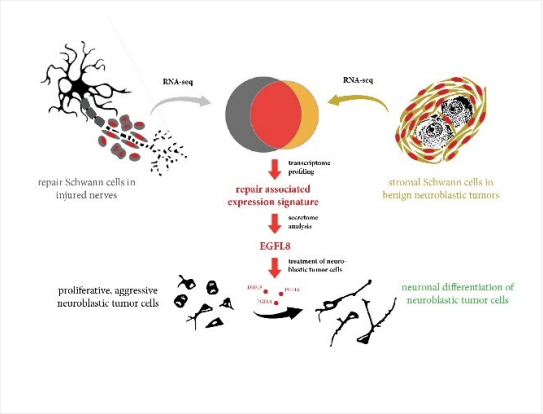
Schwann cells (SCs) possess an inherent plasticity, which allows them to acquire repair-specific functions after peripheral nerve damage. We speculate that the plastic potential of SCs also manifests in stromal SCs of benign-behaving peripheral neuroblastic tumors. Using a comprehensive transcriptomic approach, we profile ganglioneuromas and neuroblastomas, rich and poor in SC stroma, respectively, and peripheral nerves after injury, rich in repair SCs. Indeed, stromal SCs in ganglioneuromas share the expression of distinct repair-associated genes with repair SCs in injured nerves. To analyze the interaction of tumor cells and SCs in detail, we developed a co-culture model using neuroblastoma (NB) cells, derived from aggressive tumors, and primary repair-related SCs. We show that NB cells respond to repair SCs and their secretome with increased neuronal differentiation and reduced proliferation. Within the pool of factors secreted by stromal SCs and repair SCs, we identify EGFL8, a matricellular protein with so far undescribed function. We demonstrate that EGFL8 activates kinases involved in neurogenesis and induces neuronal differentiation in NB cells. These findings indicate that SCs undergo a similar adaptive response in two patho-physiologically distinct situations, peripheral nerve injury and neuroblastic tumor development. As a consequence, stromal SCs could be responsible for a benign tumor behavior by exerting repair-associated functions and that shape an anti-tumor microenvironment. Furthermore, EGFL8-mediated neuronal differentiation might hold considerable treatment possibilities for the therapy of aggressive neuroblastomas and patho-physiological conditions compromising peripheral nerve integrity.



Identification of EGFL8 as trigger of neuronal differentiation

T09-057D

Dendritic ATP release from mouse olfactory bulb granule cells induces Ca2+transients in astrocytes

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Astrocytes not only support neurons and tissue homeostasis, but also ensheath synapses with microdomains and directly interact with pre- as well as postsynaptic compartments. Adenosine triphosphate (ATP), the major messenger molecule involved in neuron-astrocyte communication, is released at axo-dendritic synapses and stimulates perisynaptic astrocyte microdomains. In addition, ATP is released from astrocytes and affects adjacent neurons and astrocytes. Here, we studied purinergic interactions between reciprocal dendro-dendritic synapses and astrocytes in the external plexiform layer of the olfactory bulb. Reciprocal synapses are composed of glutamatergic release sites of mitral cells on the one side facing GABAergic release sites of granule cells on the other side. Electrical stimulation of both mitral cells and granule cells resulted in Ca2+ transients in GCaMP6s-expressing astrocytes, whereas selective stimulation of mitral cells alone did not excite astrocytes. Stimulation-evoked Ca2+ signals in astrocytes were strongly reduced by antagonists of P2Y1 (MRS2179), and A2A receptors (ZM), while glutamatergic and GABAergic antagonists had only a minor impact. Blocking astrocytic ATP release via connexin hemichannels with carbenoxolone did not affect stimulation-evoked Ca2+ transients in astrocytes. The results indicate that granule cells, but not mitral cells release ATP that triggers an increase in Ca2+ in astrocytes. This Ca2+ increase is not amplified by ATP released from astrocytes.

T09-058D

Regulation of microglia morphodynamics by neuronal activity in the somatosensory cortex

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Microglia are the resident immune cells of the central nervous system (CNS). They widely contribute to the neuroinflammatory process, but their exact function in the non-pathologic brain remains elusive. In non-pathologic condition, microglia are characterized by highly ramified and dynamic processes that constantly probe the parenchyma. Purines appear to be important signaling molecules involved in microglia cell motility and morphology, however, the exact purpose of microglia processes motility and its potent regulation by neurons remain to be determined.

Recent data from the team indicate that neuronal activity and state of consciousness are associated with changes in microglial morphodynamics. The aim of the present study is to better understand the relationship between neuronal activity and morphodynamics of microglia cells. Because microglia motility is affected by vigilance state we will focus on adenosine, a key signaling molecule involved in sleep mechanisms and microglia chemotactism. As a first step, we intend to better characterize the interactions between synaptic activity and microglial dynamics. To that end, we used whisker stimulation in vivo to manipulate neuronal activity and monitored microglia morphodynamics by two-photon imaging. As a second step, to evaluate the contribution of the purinergic signaling pathway in the communication between microglia and neuron we administrated agonist and antagonist of adenosine receptors.

Preliminary results indicate that whisker stimulation increase calcium transients in spines from the barrel cortex. We are now evaluating the contact of microglia with spines according to their calcium activity. We also showed that the A2AR antagonist, Caffeine, abolishes sleep and decreases the amplitude of the EEG theta frequency band during wake state. Whereas the agonist CGS-21,680 had no effect on vigilance state and decreased the amplitudes of EEG delta frequency band. We now have to analyze the impact of these compounds on microglial motility and morphology. The analysis of the morphodynamics in regard to neuronal activity should allow us to determine the involvement of the adenosine pathway in the communication between neurons and microglial cells.

Overall, the present model permits the modulation of neuronal activity both physiologically and pharmacologically and will help us to determine whether adenosine is a major signaling pathway involved in the control of microglial motility in physiological conditions.

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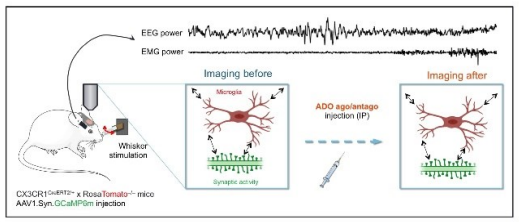

In vivo imaging of microglia morphodynamics and synaptic activity

T09-059D

Aβ-dependent GLT1 expression as neuron-glia marker for the multistage progression of Alzheimer's disease

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Extracellular accumulation of amyloid-β (Aβ) is widely recognized as a hallmark of Alzheimer's disease. In the past decade, however, experimental evidence emerged that at preclinical (asymptomatic) stages of the disease, accumulation of Aβ oligomers, appears to correlate with the onset of local networks' hyperexcitability, possibly due to dyshomeostasis of extracellular glutamate, but the interplay between Aβ production and extracellular glutamate accumulation remains unresolved. We resort to an in silico approach to characterize the putative biophysical underpinnings of Aβ-dependent modulations of extracellular glutamate.

We first consider how extracellular Aβ modifies astrocytic GLT1 expression and how it impacts glutamate time course in the peri-synaptic space. Accordingly, we develop a mathematical model for glutamate diffusion in the peri-synaptic space and uptake by astrocytic transporters. By changing Aβ concentration, we quantify the time course of glutamate and the conditions for its accumulation, considering a scaled-up version of the model that can effectively reflect the local tissue's pathophysiology. Our model predicts the existence of a threshold Aβ concentration, above which astrocytic GLT1 expression cannot prevent the accumulation of extracellular glutamate accumulation. This, in turn, promotes positive feedback on synaptic glutamate release that favors excitotoxicity and the emergence of neuronal hyperactivity.

Next, we complement our model including calcium-dependent Aβ production and glutamate release, unraveling the interaction of multiple positive feedback loops of signaling that can account for different tissue states, that go beyond the traditional dichotomy between the healthy and excitotoxic conditions. Specifically, changes in astrocytic glutamate uptake and calcium feedback on the neuronal firing can promote intermediate conditions that, if

monitored accurately, could either predict AD degeneration in advance or offer new directions for therapeutic intervention.

Our results pioneer theoretical analysis on the complex etiology of AD, supporting the notion of a multistage, progressive pathology, where transitions from one stage to the next can occur by multiple pathways that may contemplate Aβ accumulation, excitotoxicity, calcium dysregulation, or any combination thereof.

T09-060D

Necroptosis and microglial phagocytosis of extracellular vesicles as an early mechanisms involved in the disruption of afferent synaptic terminals on spinal cord motor neurons after acute peripheral nerve injury

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The disconnection of motor neuron (MN) cell bodies from their skeletal muscle targets, as occurs after a peripheral nerve section, leads to a rapid recruitment of reactive microglial cells in the affected regions of the spinal cord. In a recent study (1, 2), we have reported that the degeneration of synaptic inputs on axotomized MNs occurs in a close relationship with processes of activated recruited microglia. Synaptic terminals undergo disruption and fragmentation leading to the generation of extracellular vesicles before they are “ingested” by microglia. These observations are in contrast to those classically described under the concept of synaptic stripping, in which afferent synaptic boutons are merely detached by microglia from the surface of axotomized MNs in the absence of signs of degeneration (3).

To further explore the interactions between recruited microglial cells and presynaptic terminals on injured MN cell bodies, we have performed electron and confocal microscope analyses of these elements in axotomized MNs between 1 and 15 days after sciatic nerve transection in mice.

Between 1 and 3 days post-injury, microglial cells surrounding the injured MNs were highly phagocytic, showing an upregulation of the lysosomal marker CD68 expression. After EM examination, we did not observe any bulk engulfment of synaptic boutons by microglia. Instead, microglial cells internalized small membranous-vesicular fragments which were originated from the acute disruption of synaptic terminals. Abundant extracellular vesicles in the perineuronal space after axotomy were seen in conjunction with the expression of the necroptosis effector protein p-MLKL and, later, with the appearance of exosomal markers, such as CD9, CD63 and flotillin. Moreover, activated microglia and synaptic boutons displayed positive C1q immunoreactivity, which attained its maximum 7 days after axotomy. This suggest that a complement-mediated opsonization may also contribute to microglial-mediated synaptic elimination.

Overall, our data reveal new mechanisms by which afferent synapses are removed from acutely injured MNs. Microglial cells are actively involved in the process by eliminating fragments of damaged presynaptic terminals. Furthermore, in addition to the relevance of our data in the context of neuroinflammation and MN disease, it should

also be taken into account for understanding functional recovery after peripheral nerve injury.

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T09-062D

Astroglial $GABA_B$ receptor deletion attenuates epileptic network function *in vivo*

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Astroglial $GABA_B$ receptor ($GABA_BR$) signaling induces intracellular Ca^{2+} oscillations, triggering the release of glutamate and/or ATP. These astrocyte-derived gliotransmitters are crucially involved in neuronal network plasticity. Importantly, astroglial glutamate enhances network excitability and favors neuronal synchrony. The particular feature to release glutamate upon GABAergic stimulation places astrocytes at the interface of excitation and inhibition. In epilepsy, essential astroglial functions such as gap-junction coupling, glutamate and K^+ homeostasis are fundamentally altered. Moreover, compelling evidence shows that astroglial Ca^{2+} signaling contributes to the initiation and maintenance of epileptiform activities.Employing an intracortical kainate model of temporal lobe epilepsy (TLE), we tested the impact of astroglial $GABA_BRs$ using inducible and cell-specific knockout (cKO) mice with GCaMP3 expression. Telemetric EEG recording revealed a protective effect of $GABA_BR$ cKO during *status epilepticus*, based on a 50 % reduction in the number of seizures. To study the effect on cortical astroglial Ca^{2+} signals during propagation of epileptiform events, we combined *in vivo* 2P- Ca^{2+} imaging with ECoG recordings. During *status epilepticus*, Ca^{2+} signal architecture was generally perturbed by development of a positive correlation between signal amplitude and duration in in control and mutant mice. In $GABA_BR$ cKO, however, Ca^{2+} signal dimensions such as amplitude and duration were acutely enhanced. Moreover, epileptiform activity was associated

with high synchronization of Ca^{2+} events. Categorizing epileptiform events as seizure or spike trains, based on ECoG characteristics, we found that the association with synchronized Ca^{2+} events plays a dual role. Controls show an increased association of Ca^{2+} events with seizures, suggesting a promoting role of Ca^{2+} events, considering the overall increased epileptiform activity. Conversely, synchronous Ca^{2+} events in cKO were more often associated to spike trains, suggesting a dampening effect on spike train transition to seizures, given the generally lower seizure burden in cKO. During the latent phase, Ca^{2+} signal dimensions and architecture return to baseline levels in cKO but not in control, accounting for a more severe network disturbance. Finally, we report a protective role of $GABA_BR$ cKO in the outcome of histopathological hallmarks of TLE. $GABA_BR$ cKO animals display reduced hippocampal gliosis and a 60% reduction of granule cell dispersion incidence. Summarizing, deletion of the astroglial $GABA_BR$ has an anti-epileptic effect, whose underlying mechanisms are yet to be determined.

T09-063D

Dual effect of A1R and A2AR upon CB1R signalling in cortical astrocytes: Ca^{2+} dependent modulation of glutamate transporters

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Astrocytes express several types of receptors, namely the cannabinoid type one receptor (CB1R) and adenosine A1 and A2A receptors (A1R, A2AR). In astrocytes, CB1R activation induces Ca^{2+} mobilization from internal cellular stores modulating synaptic function[1]. Adenosine receptors have several modulatory effects, such as the control of glutamate transport, such as GLAST and GLT-1, mainly expressed in astrocytes[2]. Interactions between CB1R and adenosine receptors have been well studied in neurons[3], nevertheless nothing is known concerning this crosstalk in astrocytes. The aim of this work was to study the functional role of CB1R and A1R or A2AR crosstalk upon Ca^{2+} signaling and glutamate GLAST transporter, in rat primary astrocytic cultures.

Primary astrocytic cultures from cortex were prepared from Sprague Dawley pups (0-2 days old). Receptors interaction was evaluated by BRET (Bioluminescence Resonance Energy Transfer) and PLA (Proximity Ligation Assay) assays. Ca^{2+} signaling imaging was performed by using the fluorescent dye FURA-2AM. GLAST activity was measure by performing [3H]Glutamate uptake experiments. For statistical analysis a one-way ANOVA followed by a Bonferroni post hoc test was used and $p<0.05$ were considered to account for statistically significant differences.

We observed a physical interaction between CB1R and A1R (N=3) as well as between CB1R and A2AR (n=3-4) which was dependent on the presence of extracellular adenosine (n=3). CB1R activation with ACEA (1μM), a specific CB1R agonist, led to Ca^{2+} transients (n= 73 cells from 7 independent cultures) and a significant increase of the V_{max} of GLAST transporter ($P<0.05$, n=3). Removal of endogenous adenosine with adenosine deaminase (ADA, 2 U/mL) abolished the effect of CB1R upon GLAST (n=4-6; $p<0.05$) and induce a non-significant decrease of

CB1R-mediated Ca^{2+} transients (n=2). In the presence of ADA, the activation of A1R with the selective agonist CPA (30nM), restored the CB1R mediated effect upon Ca^{2+} signaling (N= 6-7 cells from 2 independent cultures) and glutamate uptake (N= 4). Selective activation of A2AR with CGS 21680 (30nM), diminished CB1R-mediated Ca^{2+} transients (n= 8-12 cells from 2 independent cultures) while ACEA counteracted the known diminished effect of $A_{2A}R$ in glutamate uptake (n=5).

Astrocytic CB1R activation triggers Ca^{2+} transients enhancing GLAST activity. This effect appears to be controlled by adenosine A_1R and $A_{2A}R$ since significant differences in both Ca^{2+} signaling and glutamate clearance were observed upon modulation of both adenosine receptors.

Acknowledgement

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T09-064D

Fast astrocytic calcium signals are revealed by high-frequency imaging during epileptiform activity

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Astrocytes are considered to be slow responders to neuronal function. However – using high-speed two-photon microscopy on astrocyte soma –, we previously observed that astrocytes display fast oscillatory activity during slow-wave sleep and epilepsy that matches the timing and frequency of neuronal rhythms. Therefore, we argue here that the widely unchallenged view of slow astrocytic signalling is the consequence of the usually applied low time-resolution fluorescent calcium imaging techniques; and the network-embedded astrocytes instead show a remarkably broad spectral width in Ca2+ imaging.

We evaluated fast speed calcium-imaging experiments made on astrocytes from acute hippocampal rat brain slices and simultaneously recorded neurons electrophysiologically. Astrocytes were bulk loaded with the astrocyte-specific morphological tracer SR101 and the calcium dye Oregon Green 488 BAPTA-1 AM. During low magnesium-induced epileptiform activity we observed oscillatory activity in SR101+ stratum radiatum astrocytes in the frequency of 1-10 Hz on the soma that were not present at low acquisition rate (1 Hz). Importantly, we observed frequency components in electrophysiological recordings that matched calcium oscillation frequencies - implying a causal relationship.

We conclude that at low acquisition rate, glial responses are critically undersampled, effectively resulting in information loss in the astroglial fast response range. Offline downsampling of calcium traces recorded at high acquisition rate also indicated that lowering acquisition rate not only reduces the information content of the detected astrocytic signals, but even introduces false lower frequency components. Overall, the observed mechanism endows astrocytes with fast ‘parallel computing’ capabilities in the network.

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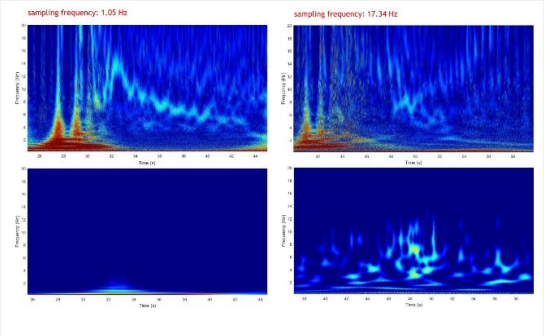

Astrocytic calcium activity can be resolved in the 10 Hz range during epileptiform activity
Simultaneous in vitro Ca^{2+} imaging of cortical astrocytes with the calcium dye Oregon Green 488 BAPTA-1 AM and LFP recording. Spectrograms of raw LFP (0.25–20 Hz) and the concurrent Ca^{2+} imaging shown at different sampling frequency (9,17 Hz; 17,34 Hz).

T09-065D

Morphological changes of astrocytes induced by olfactory enrichment

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It is known that exposure to an enriched environment is generally beneficial for cognitive performance in human and mice. The influence of enriched environment on neuronal processing and morphology has intensively been studied within the last decade. In contrast, little is known about the influence of enriched environment on astrocyte morphology and the functional consequences of that. In the present pilot study, we evaluate the impact of olfactory enrichment on the morphology of astrocytes in the mouse olfactory bulb.
Mice expressing eGFP in a subpopulation of astrocytes were exposed to a stimulating odorant mixture for 3 weeks. Following tissue clearing using XClarity and immunohistological staining, a 3D reconstruction of GFP-positive astrocytes and GFAP-positive astrocytic structures in the main olfactory bulb was made. To analyze large samples of astrocytes in three-dimensional space, semi-automatic image analysis was performed in Imaris. Using filament tracing, individual astrocytes of stimulated and un-stimulated mice were identified, and their GFAP-positive branches analyzed with respect to a variety of morphological characteristics, like total length, number of terminal points and astrocytic branch level. A closer look on structural properties was achieved by analysis of GFP-positive astrocytes, which allow for an analysis of cell volume and fine processes.
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T09-066D

SREBP2 delivery to striatal astrocytes normalizes transcription of cholesterol biosynthesis genes and ameliorates pathological features in Huntington's Disease

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Cholesterol is a multifaceted molecule essential for brain function (Dietschy & Turley, 1968). In the adult brain, cholesterol is produced locally by astrocytes and transferred to neurons through apoE-containing lipoproteins (Jurevics & Morell 1995). Disruption of brain cholesterol pathways has been linked to several neurological disorders, including Huntington's disease (HD), a genetic, neurodegenerative disorder caused by a CAG expansion in the gene encoding the huntingtin protein (Valenza & Cattaneo 2011). Brain cholesterol biosynthesis and content are reduced in several HD models (Valenza et al., 2005; 2007; 2010; Shankaran et al., 2017). The underlying molecular mechanism relies on reduced nuclear translocation of SREBP2, the transcription factor that controls the transcription of several genes involved in cholesterol biosynthesis (Valenza et al., 2015; Di Pardo et al., 2020). Work conducted in our laboratory has shown that cholesterol supplementation to the HD brain ameliorates synaptic and cognitive defects in two mouse models of HD (Valenza et al., 2015; Birolini et al., 2020).

Here, we used recombinant adeno-associated virus 2/5 to deliver exogenous SREBP2 specifically in astrocytes in order to enhance the endogenous cholesterol biosynthesis in the striatum of HD mice. We found that exogenous SREBP2 stimulates the transcription of some of the cholesterol biosynthesis genes and full restoration of synaptic transmission, reversal of *Drd2* transcript levels, clearance of mutant Huntingtin (muHTT) aggregates and rescue of behavioral deficits.

These results demonstrate that glial SREBP2 participates in HD pathogenesis in vivo, highlighting the translational potential of cholesterol-based strategies for this disease.

T09-067D

Role of astrocytic signaling in excitatory synapse maturation: implications for neurodevelopmental disorders

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Astrocytes are the most numerous glial cell types in the central nervous system. They interact with microglia and synapses, and are known to play critical roles in the progression and maintenance of neuroinflammation by responding to inflammatory mediators via G protein-coupled receptors (GPCRs) present on their surface. Furthermore, accumulating evidence suggests that postnatal inflammation during brain development is involved in the etiology of major neurodevelopmental psychiatric diseases, including autism and schizophrenia.

We hypothesize that chronic activation of astrocytic GPCR signaling during brain development triggers the production and release of synaptic modulators, that may subsequently alter excitatory synaptic transmission and plasticity.

To test this hypothesis, we used chemogenetic DREADD and AAV-based tool to selectively activate astrocytic GPCR signaling in the developing mouse primary visual cortex as a model system. These approaches were combined with immunohistochemistry, biochemistry and state-of-the-art *in vivo* electrophysiology.

We found that chronic activation of GPCR signaling in astrocytes alters the number of excitatory pre-synaptic thalamocortical terminals. The specific mechanisms underlying this phenomenon and the functional outcomes are currently under investigation.

Our study will provide a better understanding of the role of astrocytic GPCR signaling in the physiopathology of brain development, which could have implication in the treatment of some neurodevelopmental diseases.

T09-068D

Uncovering the transcriptional landscape of astrocytes highlights glial actin dynamics as important for neuronal remodeling

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It has been established that astrocytes are essential for synapse formation, maturation, and plasticity, yet their function during neuronal remodeling, and the mechanisms by which they act to regulate it, remain incompletely understood. To identify astrocytic molecules that affect axon pruning of the *Drosophila* mushroom body (MB), we profiled the expression of larval and adult astrocytes using RNA-seq. Since pruning occurs at early pupal stages, we focused on genes specifically enriched in larval astrocytes, and lower in adult astrocytes or developing neurons. By knocking down the expression of candidate genes in astrocytes, while simultaneously visualizing MB neurons, we identified several genes that were required in astrocytes for MB-axon pruning. Among these genes was Actin-related protein 2/3 complex, subunit 1 (Arpc1), which is part of the evolutionary conserved Arp2/3 complex that governs F-actin polymerization. Additionally, glial expression of other Arp2/3 complex proteins, as well as other regulators of actin dynamics, was required for MB-axon pruning. Interestingly, interfering with astrocytic F-actin dynamics did not affect their migration or gross morphology. However, we found that astrocytes silenced for Arpc1 failed to infiltrate the axonal bundle at the onset of pruning. Remarkably, decreasing axonal adhesion suppressed both pruning and infiltration defects caused by astrocytic Arpc1 knockdown, suggesting that actin-dependent astrocytic infiltration is a key step in the execution of axon pruning. Thus, we have identified a yet unknown actin-based mechanism by which astrocytes affect neuronal remodeling. Insights from this study can expand our knowledge regarding the significance of neuron-glia interactions during proper brain development.

T09-069D

Characterising the functional role of the myelinic channel

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Introduction: The insulating properties and function of CNS myelin are well characterised but a comparatively more recent development is the understanding that myelinating cells support the function and survival of myelinated axons. Studies have shown that myelinating oligodendrocytes can provide glycolytic products to fuel oxidative phosphorylation in axonal mitochondria. However, little is known about how axons signal their support requirements to post-myelination oligodendrocytes (PMO) or how glial factors reach the glial-axonal junction in the CNS. Here, we propose that the myelinic channel is the route by which this interaction occurs. The myelinic channel is a cytoplasm-filled space that runs around the inner and outer tongue of the myelin wraps, and paranodal loops. We also propose that neuronal electrical activity communicates (directly or indirectly) with the PMO, modulating the glial cell's function.
Methods: We generated 'myelinating' cell cultures from E13 mouse embryos expressing tdTomato fluorescent protein in the oligodendroglial cytoplasm and EOS2-tagged oligodendroglial peroxisomes, which, in conjunction with fluorescent microscopy and live imaging were used to investigate our hypothesis.
Results: We show that peroxisomes are located throughout the myelinic channel, as delineated by tdTomato, and that these myelin peroxisomes are motile, moving at rates compatible with motor protein-dependent transport on microtubules.To determine if myelin peroxisome transport is microtubule-dependent, we disrupted the microtubule dynamics pharmacologically. This led to a decrease in peroxisome motility, suggesting that peroxisomes move via the microtubule network in myelinating oligodendrocytes. Using pharmacological modulators, we found that neuronal electrical activity, a surrogate for axon energy consumption, modulated the motility of myelin peroxisomes. These data provide functional evidence that the axon's electrical activity induces functional changes (directly or indirectly) in the PMO, suggesting further that the myelinic channel acts as a transport route between the oligodendrocyte soma and the glial-axonal junction.**Conclusions:** Our data provide evidence that peroxisomes reside in the myelinic channel and goes some way to identify the functional role that the myelinic channel plays in supporting the axon, including evidence of bidirectional communication between the two cell types. Further characterisation of the myelinic channel will aid in our understanding of the processes that lead to the degeneration of axons following oligodendrocyte injury in diseases such as multiple sclerosis.

T09-070D

The Role of Neuronal NIPP1 in Mediating Neuron-Oligodendrocyte Signaling to Promote Myelin Biogenesis

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Myelination of axons enables rapid transduction of action potentials, timely communication between neurons in different brain regions, and neurotrophic support for axons. Neuronal activity positively regulates CNS myelination, especially in the cortex and corpus callosum. Many studies have focused on the possible mechanisms by which neuronal activity could regulate CNS myelination. For example, neuron-released trophic factors, axon caliber, and/or synaptic transmission between neuron and oligodendrocyte cells have all been shown to influence oligodendrocyte lineages and myelination. However, how nuclear factors regulating neuronal activity in CNS myelination has not been explored. Nuclear inhibitor of protein phosphatase 1 (NIPP1) is a known regulator of gene expression and has been shown to play roles in many physiological processes such as stem cell proliferation, and cancer cell progression, but whether it plays a role in CNS function is not clear. In the present study, by using a conditional knockout (KO) mouse model, we found that KO of NIPP1 in neuroprogenitor cells (NPCs) led to severe myelination deficit in the CNS. Proper myelination was examined by the protein expression level of key myelin constituent protein, myelin basic protein (MBP), the percentage of axons having myelin sheath, g-ratio as well as direct measure of action potential propagation in optic nerve. Interestingly, we found that KO of NIPP1 in neurons, but not in oligodendrocyte-lineage cells, recapitulated the CNS myelination deficit when NIPP1 was knocked out in NPCs. Moreover, we found that neuronal excitability is decreased in both pyramidal neurons in the mouse hippocampal CA1 and cortical layer V regions. Our data thus suggest that NIPP1 regulates neuronal activity and modulates CNS myelination.

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T09-071E

Ceruloplasmin deficiency is associated with proteomic changes of oligodendrocytes and neurons in absence of overt changes of myelin and myelinating cells in mice

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Ceruloplasmin (Cp) is a multicopper oxidase with ferroxidase properties with important functions in the transport and oxidation of iron. The brain is rich in iron, with oligodendrocytes being the main iron-containing cell type, and patients with aceruloplasminemia develop neurological symptoms as one of the first severe symptoms due to intracellular iron accumulation. In this study, we used proteomics and a Cp knockout mouse ($Cp^{-/-}$) (B6N(Cg)-$Cp^{tm1b(KOMP)Wtsi}$/J) to investigate the effect of Cp on the brain proteome in adult 4-5-month-old mice. Proteomics was corroborated with Luxol fast blue (LFB) staining of myelinated tracts and quantitative PCR for changes in gene expression. The proteomics results showed that $Cp^{+/-}$ mice had more regulated proteins, and more proteins with post translational modifications (PTMs) than $Cp^{-/-}$ mice. Most regulated proteins had a neuronal or an oligodendroglial origin. PTMs were identified in myelin associated glycoprotein (MAG) and Nogo-A, which are binding partners for the reticulon 4 receptor (RTN4R). The intensity of the LFB staining indicated no gross effects of

Cp on the myelin. Additionally, the density of Olig2^{+k}oligodendrocyte lineage cells in the corpus callosum was unaffected by Cp, in 9-day-old pups. In line with these data, no significant changes were detected in the transcript level of oligodendrocyte lineage and myelin genes. Our findings suggest that Cp affects the close interaction between oligodendrocytes and neurons, however in a way that does not lead to gross morphological, structural or cellular changes of the CNS in the normal adult mouse.

T09-072E

The role of potassium in axon-glial signaling and metabolic coupling

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Myelinating oligodendrocytes (OLs) are a crucial element of the vertebrate nervous system. Not only do they shape axonal conduction speed but they were also highlighted to maintain long-term axonal integrity by providing metabolic support to the axons they ensheath. However, little is known about how OLs sense axonal spiking activity and how axon-glial metabolic coupling is regulated. Here, we combine electrophysiological recordings and two-photon FRET imaging of acutely isolated optic nerves to study activity-dependent Ca^{2+} dynamics in OLs and metabolite fluxes in myelinated axons. The stimulus-induced Ca^{2+} response in OLs was mediated by facilitation of Ba^{2+}-sensitive, inwardly rectifying K^+ channels. Apart from being crucial for K^+ clearance we demonstrate that oligodendroglial Kir4.1 activity also serves metabolic support functions to myelinated axons. We found reduced basal axonal lactate levels and stimulus-evoked lactate surges in OL-specific Kir4.1 knockout (Kir4.1 cKO) mice. These perturbations in axonal lactate homeostasis were not due to deficits in axonal glycolysis nor alterations in mitochondrial respiration. Moreover, Kir4.1 cKO mice developed late onset axonal pathologies likely due to an impaired axonal energy homeostasis. Taken together, our study provides a working model of how OLs sense axonal activity and regulate axonal energy metabolism through a Kir4.1-mediated mechanism.

T09-073E

Postnatal expression of the AQP4, TRPV4 and Cx43 in the CNS: potential differences between Muller cells and astrocytes

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Aquaporin-4 (AQP4) is the Central Nervous System (CNS) water channel playing a critical role in cell and extracellular space homeostasis and AQP4 altered expression appears to be involved in several pathological conditions such as brain edema, stroke and tumors. AQP4 is expressed at the glial endfeet surrounding the blood vessels together with the swelling sensitive Transient Receptor Potential Vanilloid 4 (TRPV4) and the gap junctional protein connexin-43 (Cx43). AQP4 and TRPV4 control the water and ion homeostasis in glial cells in either brain or retina. Furthermore, the astroglia syncytia allows water and ions to be cleared from neuropil to the vascular side of the glial compartment through gap junctions, predominantly constituted by Cx43. The aim of this study has been to investigate the expression of TRPV4 and Cx43 during development in both brain and retina, and the effect of AQP4 deletion on their expression pattern. In rat and WT mouse, TRPV4 was upregulated at postnatal day (PD) 7 and then downregulated during development in both brain and retina. AQP4, Cx43 and the glial cell marker GFAP showed a similar temporal pattern increasing their expression during development. Contrary to GFAP and AQP4, immunoblot and confocal microscopy analysis performed in neuronal stem cells (neurospheres) induced to differentiate, showed that TRPV4 was strongly expressed within undifferentiated spheres rather than into differentiated neurons or glia. The analysis of AQP4 KO mice revealed no difference in Cx43 expression between WT and AQP4 KO mouse brain and retina, but a higher level of Cx43 phosphorilation at PD7 in AQP4 KO retina. Interestingly, TRPV4 expression was strongly downregulated in AQP4 KO retina from PD7 to adult while no alteration was found in AQP4 KO brain. No TRPV4 altered expression was found in AQP4 siRNA treated astrocyte primary cultures. Collectively, we demonstrate a distinct temporal expression pattern for Cx43 and TRPV4 in brain and retina probably related with their different function during development. Our findings suggest a strong impact of AQP4 deletion on TRPV4 expression in the retina and not in the brain suggesting a different mechanism of TRPV4 and AQP4 functional interaction in astrocytes and Muller cells.

T09-074E

Microglial and neurogenic alterations in hypothalamus due to acute stress

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Microglial cells are an important glial population known to be involved in several biological processes such as stress response. These cells engage an activated state following a stress insult that may lead to nervous tissue damage, including new cell generation impairment. This has been widely studied in regions with notable neurogenesis such as de hippocampus, however, the effect in other regions with fewer yet relevant neurogenesis remains partially unknown. One of them is the hypothalamus, a key vegetative control center playing an important role in stress response. Moreover, most of the stress models studied concern neuroinflammatory and neurogenic

changes due to a chronic stressor but not a single stress event. Given the repercussion of these processes alone, it would be interesting to elucidate the relationship between microglial response, hypothalamic neurogenesis, and acute stress.

Therefore, this project focuses on studying acute stressed C57BL/6J mice, both at the histological and molecular level. An intense stressor combining water immersion and movement restriction was performed. Using immunohistochemical and molecular analysis with Luminex, we could analyze microglial distribution and morphology, neurogenesis, and inflammatory environment in the hypothalamic parenchyma. Three regions related to stress were studied: the paraventricular, ventromedial and arcuate nucleus.

Results pointed to a wider soma microglial morphology near the arcuate nucleus, particularly in the ventromedial region. This state was enhanced by acute stress, but not in stress priming. Regarding newborn cells, despite overall proliferation being decreased, cell survival was not significantly different. Moreover, changes in young neuron (DCX+) presence were not observed in the aforementioned hypothalamic nucleuses but it was highly decreased in the periventricular zone. As a matter of fact, these changes were microglia-mediated according to a statistical mediation analysis. A possible explanation to this could be the peak in IL-6 levels within the hypothalamus observed 1 h after stress treatment, which returned to basal levels after 24 h. Other inflammation chemokines were not affected, and further stress priming did not affect the parameters studied.

As a conclusion, this study supported the particularly relevant effect of acute stress in hypothalamic microglia, which leads to a reduction in cell proliferation, probably due to inflammatory chemokine release into the parenchyma.

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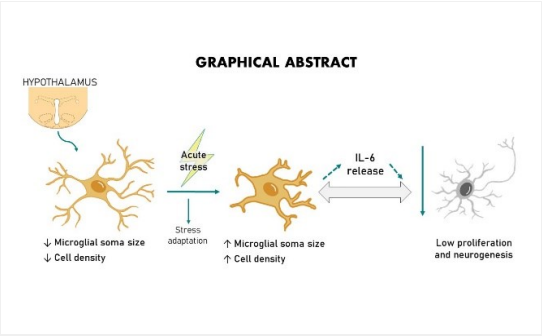

Graphical Abstract
Microglial and proliferation alterations in the hypothalamus due to acute stress. A microglial remodelling in several nucleuses of the hypothalamus is observed under acute stress. This variation involves morphologic changes in microglia (wider cell soma and shorter processes), which could statistically mediate the reduction in cell proliferation in the region. A peak observed in tissue IL-6 one hour after the stressor could be related to these changes. Moreover, an adaptation to the stressor was observed as no signifiant changes were found after a stress priming process.

T09-075E

Modulation of L-lactate release by astrocytic $GABA_B$ receptors and cyclic AMP signalling *in vitro*

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Astrocytes are crucial for the maintenance of the excitation/inhibition balance of neural circuits and therefore must sense and respond to local neuronal activity. Their regulation of glutamatergic transmission is currently better understood than that of GABAergic. While $GABA_B$ receptor activation is generally associated with Gi/o protein signalling, previous work in astrocytes suggested an additional IP_3-dependent pathway that leads to intracellular Ca^{2+} oscillations.

In this study, we set out to investigate whether the $GABA_B$ receptor agonist baclofen modulates production and release of the gliotransmitter L-lactate (lactate) in dissociated primary astrocyte cultures. Specifically, we focused on the cyclic AMP (cAMP) dependent pathway which has been linked glycogenolysis. We used the FRET-based nanosensor Laconic to monitor intracellular lactate dynamics and a fluorimetric L-Lactate assay (EnzyFluo) to measure lactate release.

We found that baclofen (10μM-100μM) inhibited production of lactate (88±24.9% ; n=15) in unstimulated astrocytes, and that lactate production and release under these conditions was partially glycogen-dependent. However, an inhibitory effect of baclofen on basal lactate release was not detectable, presumably since constitutive lactate production is low.

Activation of adenylyl cyclase by NKH 477 (6-[3-(dimethylamino)propionyl]-forskolin; 0.3μM-30μM) resulted in lactate mobilisation in a concentration dependent manner. Interestingly, the increase in Laconic signal peaked at 3μM NKH, but lactate release from the cells was maximal at 10μM NKH, suggesting potentially differential effects of cAMP signalling on lactate production and release mechanisms.

The cAMP driven lactate production was inhibited by blocking glycogenolysis with DAB (1,4-Dideoxy-1,4-imino-D-arabinitol; 500μM), by inhibition of PKA activity with H89 (10μM), or by activation of $GABA_B$ receptors with baclofen (100μM).

This work highlights complex cross-talk between cAMP and Ca^{2+} signalling pathways in regulation of lactate release from astrocytes.

Acknowledgement

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T09-076E

Characterization of microglia and synapses in the early brain of an AD mouse model

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Microglia are the resident macrophages and immune cells of the brain. Besides their immunity role, microglia also orchestrate several processes, critical for proper neuronal functioning. They are implicated in a variety of tasks ranging from removal of neuronal debris to specific refinement of synaptic terminals, contributing to maturation and monitoring of neuronal circuits. In neurodegeneration, such as in Alzheimer's disease (AD), microglia display excessive synaptic phagocytosis, at least at early stages, leading to pathological synapse loss. Most of the studies have been conducted to characterize the role of microglia in overt pathological stages. However, whether synaptic pruning by microglia in AD brains initiates long before, e.g. during brain development, has received little attention. To address this question, we have investigated microglia and synapses in the early brain of an AD mouse model, using transgenic mice overexpressing the human Amyloid Precursor Protein (hAPP) carrying the Arctic and Swedish mutations (ArcAβ). We used biochemical and histochemical complementary approaches, to assess microglial morphology and density, as well as dendritic spine density and synaptic markers, in the hippocampus of two weeks and one-month-old mice, to identify early alterations. In addition, lipidomic profile of hippocampal synaptosomes was assessed to reveal whether hAPP overexpression affects synapses during brain development. Overall, these data will inform us whether microglial and synaptic alterations are already present at early stages in the brain of an AD mouse model, thus providing susceptibility to neurodegeneration later in life.

T09-078E

Astrocyte Expression of Synapse-regulating Genes is Developmentally Controlled by Neuronal and Astrocyte Activity

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Astrocytes are important regulators of neuronal synapse development and function. In the rodent cortex neurons are arranged in layers, each with stereotyped synaptic connectivity. Cortical astrocytes produce several synapse promoting factors, however, what regulates their regional and temporal expression during development is unknown. Here we use RNA sequencing, genetic mouse models, immunohistochemistry and in situ hybridization to determine the expression profile of astrocyte synapse promoting genes, and their regulatory mechanisms in the

developing mouse visual cortex[1]. This analysis identified astrocyte signals that show differential temporal and layer-specific expression. The observed expression changes occurred mainly between post-natal days (P) 7 and 14, a time between synapse initiation and maturation. We focused on factors that promote expression of AMPA receptors (AMPARs), crucial components of active excitatory synapses, such as glypican 4 (Gpc4) and chordin like 1 (chrdl1). AMPAR subunit composition undergoes a developmental switch, whereupon GluA1 is prevalent in immature synapses, while GluA2 marks a mature synapse. Gpc4, which promotes expression of GluA1 is highly expressed at P7, and is downregulated at P14 specifically in layer (L) 1, suggesting a role in synapse initiation. Chrdl1, which promotes synapse maturation by regulating expression of GluA2, peaks at P14 in the upper layers. These temporal and regional changes are regulated by visually evoked neuronal activity, as they are absent in mice lacking glutamate release from thalamo-cortical projections, resulting in delayed synapse maturation. Further, expression of synapse regulating genes and synaptic development are also altered when astrocyte signaling is blunted by diminishing calcium release from intracellular stores, as evident from mice lacking the Ip3r2 gene. Single nucleus RNA sequencing identified astrocytic genes from various functional categories, that are regulated by neuronal and astrocyte activity, along with multiple genes that show layer-specific enrichment. These findings provide insight into neuron-astrocyte interaction as it occurs at the level of the distinct cortical connections, and constitute an important frame work for future studies of astrocyte and synapse development in the cortex.

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T09-079E

Mechansims of cerebellar microglial dynamics

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Synaptic plasticity allows the central nervous system (CNS) to incorporate new sensory experiences and information, and its disruption is associated with many neurological and psychiatric disorders. Much recent work has focused on the contribution of non-neuronal CNS cells, especially microglia, the innate immune cells of the CNS, to synaptic plasticity. Though classically thought of in their immune capacities, microglia are vital to many homeostatic and developmental processes, including synaptic plasticity of nascent and adult neuronal networks. Despite the emerging consensus that microglial dynamics are critical to brain function during physiological as well as pathological conditions, it is unclear whether these microglial roles and their underlying mechanisms are universal or differ between brain regions. There is a growing body evidence to suggest microglia exhibit a high degree of regional specialization; existing on a continuum from homeostatic (cortex, striatum) to immune vigilant (cerebellum) even in the absence of pathological stimuli. Indeed, microglia in the cerebellum represent a distinct population, exhibiting unique transcriptional and epigenetic profiles, along with distinct functional properties, such as being more phagocytic, morphologically less ramified and less densely distributed. As a consequence, cerebellar microglia survey less of the brain parenchyma than cortical microglia, but compensate for this by undergoing frequent somatic translocations under homeostatic conditions, a phenomenon not observed in cortex.

Despite these differences, cerebellar microglia maintain common microglial functions, exhibiting a robust injury response and dynamic interactions with surrounding neural elements. Two pathways of particular interest to cortical microglial mediated synaptic plasticity include noradrenergic signaling through the β_2 adrenergic receptor (β_2-AR) and purinergic signaling through the P2Y12 receptor, both of which have been shown to be critically involved in microglial roles in synaptic remodeling and rapid chemotaxis to sites of injury. We used time-lapse in vivo imaging of adult CX3CR1-GFP mice through a chronic cranial window preparation to visualize cerebellar microglial dynamics while β_2-AR and P2Y12 signaling were manipulated. We found that β_2-AR stimulation reduced microglial surveillance in both the cortex can cerebellum but had no effect on cerebellar soma migration. Lastly, we demonstrated that P2Y12 deficiency did not alter the response of cerebellar microglia to focal injury, unlike what is seen in cortical microglia. These findings demonstrate that cerebellar microglia likely use a distinct set of molecular cues to guide their dynamic responses in both homeostatic and pathological conditions.

T09-080E

Fast voltage fluctuations in cortical astrocyte microdomains shape glutamatergic neurotransmission.

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Astrocytes remove extracellular glutamate, the primary excitatory neurotransmitter in the CNS, via their expression of the excitatory amino acid transporters (EAATs) GLT1 and GLAST[1]. Glutamate uptake from the extracellular space is a highly dynamic process. Our recent work has shown that EAAT-mediated glutamate uptake undergoes rapid, localized inhibition in response to neuronal activity[2]. The mechanisms governing activity induced EAAT inhibition, however, are poorly understood. Relative enrichment of astrocytic EAATs in the distal astrocytic leaflets[3] and several lines of evidence under-lining the inhibition of EAAT function at depolarized membrane potentials[4-8] led us to ask the following questions: Does neuronal activity depolarize astrocytic microdomains, and if so, is it sufficient to inhibit EAAT activity? Using a combination genetically encoded voltage sensors (GEVIs), glutamate sensors (iGluSnFr) and single cell electrophysiology, we provide evidence that neuronal activity depolarizes astrocytic microdomains. The magnitude of these depolarizations is sufficiently large to facilitate EAAT inhibition. Our ongoing investigations explore firstly, several factors that lead to astrocytic microdomain depolarization and test its causal relationship with EAAT mediated glutamate uptake. Secondly, we study astrocyte microdomain heterogeneity and activity induced plasticity in glutamate clearance and astrocytic membrane depolarization. Our study concludes that changes in astrocytic membrane potential locally inhibit glutamate transporter shaping extracellular glutamate dynamics and synaptic function.

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T09-081E

Role of astrocytic $GABA_B$ receptors on γ-hydroxybutyric acid-induced absence seizures

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Absence seizures are non-convulsive epileptic events characterized by brief losses of consciousness and unresponsiveness to stimuli. They are commonly observed in pediatric or juvenile epilepsies. The weak $GABA_B$ receptor agonist γ-hydroxybutyric acid (GHB) mimics generalized spike and slow-wave discharges (SWDs) characterizing absence epilepsy and is therefore used as a pharmacological model of absence seizures. Given the role of astroglia in modulating and sustaining neuronal synaptic activity and their involvement in many different pathological scenarios, we investigated the role of astroglial $GABA_B$ receptors in the genesis and progression of GHB-induced absence seizures. We took advantage of the CreERT2-LoxP system to induce time-controlled astrocyte-specific gene deletion of the $GABA_{B1}$ subunit resulting in lack of functional $GABA_B$ receptors in astrocytes. *Ex vivo* GHB administration in presence of the voltage-gated Na^+ channel blocker tetrodotoxin induced longer intracellular Ca^{2+} signals with higher amplitudes in astrocytes expressing the genetically encoded Ca^{2+} indicator GCaMP3 and imaged by two-photon laser-scanning microscopy (2P-LSM). Mice lacking functional astroglial $GABA_B$ receptors showed unaltered Ca^{2+} signals compared to baseline. *In vivo* GHB induced highly synchronous Ca^{2+} waves in cortical astrocytes immediately after intravenous injection in control animals. On the contrary, Ca^{2+} signals after GHB injection were lower in number in conditional knock-out animals (cKO). Moreover, loss of astroglial $GABA_B$ receptors resulted in the alteration of GHB-induced dose-response assessed through *in vivo* telemetric electroencephalographical recording of brain activity and behavioral video monitoring. Taken together, these results suggest a role of astroglial $GABA_B$ receptors in the mechanisms underlying GHB-induced absence seizures. This makes this receptor a promising target for the treatment of absence epilepsy, which is still effective in only half of the patients.

T09-082E

Gestational 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Exposure Induces Long-lasting Defects in Microglial Function

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Microglia, the immune cells of the brain, have a canonical role in regulating neuropathological processes. Recently, they have been implicated as regulators of neurophysiological processes such as learning and memory as well. Given this dual role as immune responder and neurocircuitry modulator, microglia are well positioned to translate toxic stimuli into defects in neurocognitive function. Exposure to prevalent environmental toxicants during gestation, which is a particularly vulnerable time for brain and microglial development, could be especially disruptive. However, it remains unknown whether and how exposure to such external stressors during gestation causes neuroinflammation which could negatively impact microglial functions *in vivo*, thereby leading to neurocircuitry defects. 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a highly toxic and widely distributed environmental contaminant with high potential for human exposure, as it bioaccumulates up the food chain and can persist in the human body with a half-life in the range of 10 years, making it a significant human health risk. Gestational exposure to TCDD has been demonstrated to both cause neurological changes with synaptic alterations and induce a rapid response in microglia *in vitro* through the aryl hydrocarbon receptor. Given this, it is important to understand whether and how TCDD-induced neuroinflammation negatively impacts microglial functions *in vivo* in regulating healthy neurocognitive processes. Here, we examined the impact of gestational TCDD exposure on microglial characteristics in the visual cortex of the mouse, where microglia have been demonstrated to be critically important for visual system plasticity. We found that gestational exposure to TCDD induced a mild pro-inflammatory environment, with an upregulation of microglial pro-inflammatory markers, but no change in microglia density, distribution, morphology, and motility. However, TCDD-exposed microglia responded more strongly to a secondary insult later in life in the form of an acute laser ablation. Taken together, these results suggest that gestational exposure to TCDD may lead to microglial priming and dysregulate the ability of microglia to appropriately respond to relevant stimuli. We next assessed whether these defects could be rescued by depleting the TCDD-exposed microglia with a CSF1R-inhibitor and allowing the microglia to repopulate. We found that this microglial renewal rectified both the pro-inflammatory environment and normalized microglial responses to injury in adulthood. Our results suggest the possibility that microglial functional defects resulting from gestational environmental insults can be treated with microglial repopulation.

T09-083E

Astrocytic modulation of cortical synaptic plasticity: Integrating biological knowledge and computational modeling

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Astrocytes have been shown to control synapse development and function (Allen and Eroglu, 2017). Accumulating evidence also indicate that astrocytes can modulate long-term synaptic transmission and plasticity during postnatal development. However, the biochemical and biophysical mechanisms responsible for these modulations are not fully understood. The mechanisms involved do not only depend on the developmental stage of an animal but also on the brain area and neural circuitry in question. We have analyzed previously published computational models that involve some of these mechanisms (Manninen et al., 2018) and also developed a new computational model of astrocyte-neuron interactions at a layer 4 to layer 2/3 synapse in somatosensory cortex during postnatal development (Manninen et al., 2020). The synapse model is built based on a multitude of data from molecular, biochemical, and electrophysiological experiments in rodent somatosensory cortex. In brief, we showed that **1)** endocannabinoids released from the postsynaptic neuron increase astrocytic calcium concentration, similarly to Min and Nevian (2012), **2)** the increase in astrocytic calcium concentration can induce exocytosis of glutamate, and **3)** the astrocytic glutamate exocytosis activates presynaptic N-methyl-D-aspartate receptors and calcineurin signaling as well as influences synaptic properties. Using the computational model, we were able to make predictions about the dynamics of astrocyte-mediated molecular mechanisms underlying synaptic plasticity and link complex biochemical signaling networks at the presynaptic, postsynaptic, and astrocytic sites to the plasticity changes in a synapse. In the present study, we studied the model to mimic the motility of fine astrocyte processes during synaptic activation, particularly emphasizing long-term plasticity changes (Bernardinelli et al., 2014; Sakert et al., 2017). In this context it is of interest to analyze in detail the roles of extracellular glutamate. We explored in silico the amount of glutamate spillover required to induce spike-timing-dependent long-term depression (t-LTD) and the wide time window of t-LTD both with astrocyte activation in a tripartite synapse and without astrocyte activation in a situation when a fine astrocyte process retracts from the synapse during a physiological phenomena, such as learning, injury, or brain disease. Our simulations confirmed that during postnatal development astrocytes influence synaptic computations as well as biochemical processes for plasticity, a precondition for learning and memory. Computational studies of synaptic functions tightly integrated with biological knowledge and data promote development of new hypotheses that can be tested experimentally.

Acknowledgement

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T09-084E

Neuron-glia co-culture models of cell type specific lipid perturbations relevant to Parkinson's disease and Lewy body pathobiology

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Mutations in the lipid metabolism gene beta glucocerebrosidase *(GBA)* and polymorphism in the lipid transporter *APOE* have been identified to be significant risk factors for Lewy body disorders (LBD). Overall changes in sphingolipid levels and cell type-specific neutral lipid changes have been reported in Parkinson's disease (PD), a related LBD, suggesting a potential pathological role for lipid dyshomeostasis in the etiology of neurodegenerative disorders (Rocha EM et al., 2015; Huebecker M et al., 2019; Brekk O et al., 2020).

To investigate the effect of lipid pathway dysregulation mediated by GBA and APOE on the morphology and function of neurons and glial cells in LBD, this project aims to utilize a 2D co-culture system with cortical neurons, astrocytes and microglia-derived from primary rodent cells and human induced pluripotent stem cells with mutations in *GBA* and *APOE* genes. To identify disease relevant cellular insults that alter intracellular neutral lipid levels, primary rat-derived astrocytes and microglial cells in monoculture were treated with varying concentrations of mitochondrial depolarizing agents valinomycin and rotenone, TLR3 and 4-dependent inducers of inflammation Poly(I:C) and LPS, and an inhibitor of GBA enzyme activity Conduritol b epoxide (CBE). The cell cultures were analyzed using the neutral lipid probe BODIPY. Elevated BODIPY signals were observed in both astrocytes and microglial cells upon rotenone and valinomycin treatments. Murine macrophage cells co-treated with rotenone and the acyl CoA synthetase inhibitor TriacsinC showed reduced BODIPY signal intensity from lipid droplets (LDs), thus confirming that LD induction in response to rotenone is the result of *de novo* synthesis of triacylglycerol. BODIPY induced changes were also seen in LD analysis of astrocytes and microglia following exposure to Poly(I:C), LPS and CBE. These data show that cellular stressors relevant to PD and LBD induce neutral lipid changes in astrocytes and microglial cells. Further investigation into how these cellular responses vary in neuron-glia co-cultures and the mechanisms that lead to these changes will help identify cell-cell interactions between neurons and glia in LBD and PD.

T09-085E

Astrocytes-derived Extracellular Vesicles in motion at the neuron surface: involvement of the prion protein

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Extracellular Vesicles (EVs) shed from the plasma membrane of astrocytes play an essential role in intercellular communication. Exposing adhesion receptors, they can interact with target cells and deliver complex signals. It has been shown that EVs also cover a crucial role in the spreading of pathogens in neurodegenerative disorders, but almost nothing is known about how EVs interact with neurons at preferential sites and how EVs reach these sites in the extracellular microenvironment exploiting neuronal connections.

To investigate the interaction of EVs with the plasma membrane of neurons, EVs released from cultured astrocytes and isolated by differential centrifugation were added to the medium of cultured hippocampal neurons. Using optical manipulation, single EVs in suspension were trapped by an infra-red laser collimated into the optical path of the microscope and delivered to neuron surface. The EV-neuron dynamics were monitored by collecting brightfield images.

After contact, EVs efficiently adhered to the neuronal cell body, dendrites and axons. Surprisingly, after adhesion a large fraction of EVs moved on the surface of neurites in both retrograde and anterograde directions. Sometimes, EVs explore dendritic protrusion and during their path stop at preferential sites promoting the formation of new filopodia.

Interestingly, we found that EV movement is highly dependent on neuron energy metabolism and is driven by the binding of the EV to a surface receptor that slides on neuronal membrane, thanks to myosin-dependent cytoskeleton rearrangements. Indeed, inhibition of neuron actin filaments rearrangements with cytochalasin D or blebbistatin reduced EVs in motion, revealing that neuronal actin cytoskeleton is implicated in EV-neuron dynamics. Interestingly, the use of prion protein (PrP)-coated synthetic beads and PrP knock out EVs/neurons point at vesicular PrP and its receptor(s) on neurons in the control of EV motion on the plasma membrane.

Unexpectedly, we found that EVs can contain actin filaments and ATP and have an independent capacity to actively move at the neuron surface in an actin-dependent manner. Our data support two different way of EV motion. First, EV displacement could be driven by the binding with neuronal receptors linked to the actin cytoskeleton. Second, EVs could possess motile ability like that produced by actin in cells and move along a gradient of neuronal receptors.

Here we show for the first time that astrocytic EVs exploit vesicular PrP and its neuronal receptors to passively/actively reach their target sites on neurons.

T09-086E

Efficient oligodendrogenesis and remyelination are neuroprotective

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Multiple sclerosis is characterized by inflammatory lesions resulting in the loss of myelin and oligodendrocytes. However, the loss of axons drives accumulating disability. Most lesions typically fail to efficiently regenerate myelin sheaths leaving the majority of axons chronically demyelinated. The failure to remyelinate is in large part because oligodendrocyte progenitor cells (OPCs) are unable to fully differentiate and generate new oligodendrocytes depriving axons of critical support. However, it remains unclear if a failure to remyelinate directly drives ongoing axonal and neuronal degeneration. This question has been challenging to answer in animal models of MS which have relatively efficient remyelination. We developed two tamoxifen-inducible conditional knockout mouse lines that

delete *Myrf,* a critical transcription factor for myelination. The *Myrf*$^{fl/fl}$;PLP-CreERT line (*Myrf*$^{\Delta iPLP}$) induces loss of *Myrf* in just mature oligodendrocytes. The *Myrf*$^{fl/fl}$;Sox10-CreERT2 line (*Myrf*$^{\Delta iSox10}$) induces loss of *Myrf* in both mature oligodendrocytes and OPCs. *Myrf*$^{\Delta iPLP}$, *Myrf*$^{\Delta iSox10}$, and *Myrf*$^{fl/fl}$ controls were administered tamoxifen (TAM) at eight weeks of age and a subset of mice received EdU in their drinking water from 1 to 10 weeks post-TAM. Extensive demyelination is observed throughout the CNS by ten weeks post-TAM in *Myrf*$^{\Delta iPLP}$ mice, with 44.7 ± 9.5% of axons myelinated in the optic nerve. 86 ± 1.8% of these myelin segments have thin myelin (g-ratio > 0.85), indicative of active remyelination. New oligodendrocytes are produced in *Myrf*$^{\Delta iPLP}$ mice and there is substantial remyelination by 20 weeks post-TAM, culminating in 70.0 ± 2.2% axons myelinated in the optic nerve. In contrast, *Myrf*$^{\Delta iSox10}$ mice are unable to produce many new oligodendrocytes indicated by the lack of EdU+-labelled oligodendrocytes and are unable to express the new myelin/oligodendrocyte marker BCAS1. Severe and sustained demyelination is observed in the white matter of *Myrf*$^{\Delta iSox10}$ mice, with just 1.8 ± 0.6% of axons remaining myelinated within the optic nerve at 10 weeks post-TAM. *Myrf*$^{\Delta iSox10}$ have worsened motor function relative to *Myrf*$^{\Delta iPLP}$ mice from eight weeks post-TAM onwards. Outright axon loss was only detected in the optic nerves of *Myrf*$^{\Delta iSox10}$, indicating that the early remyelination seen in *Myrf*$^{\Delta iPLP}$ mice protects axons. Apoptotic retinal ganglion cells, which project their axons through the optic nerve, were observed at a higher rate in *Myrf*$^{\Delta iSox10}$ relative to *Myrf*$^{fl/fl}$ controls. Together, these data directly demonstrate that efficient remyelination is neuroprotective. These cell-selective models will be useful to elucidate how neurons respond to demyelinating insult and to determine the mechanisms by which remyelination failure triggers axonal degeneration.

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T09-087E

Astrocytic modulation of neuronal rhythmicity through K+ uptake in the spinal locomotor network.

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The CNS contains two major types of cell populations, neurons and glia. While glia has been for a long time overshadowed by neurons, their more electrically-excitable and popular partners, the development of improved tools allowed bringing new sights on the neuro-glia crosstalk. Astrocytes, the prevailing subtype of glia in the CNS, are highly inter-connected and intimately communicate with neurons to ensure the development, maintenance, and function of the CNS. In particular, they modulate the neuronal excitability by changing the concentration of potassium ions in the extracellular environment, a process called K+ uptake.

Thus, this study aims to **determine the astrocytic modulation of spinal locomotor oscillations.** We hypothesize that astrocytic **potassium (K^+) uptake** is crucial for tuning the **locomotor central pattern generator** (CPG) in an activity-dependent manner. In this CPG, K^+ increase promotes rhythmogenesis and K^+ homeostasis is mainly mediated by astrocytes. Though it is stated that astrocytes process neuronal information and displaycalcium signals, there is still uncertainty about how neurons compute information from astrocyte network. In this study, by using transgenic mice, we determined how and to what extent the spinal CPG astrocytes (i) display distinct morpho-functional properties (ii), are active during rhythmic oscillations, and (iii) influence the locomotor pattern.

We demonstrated that (1) astrocytic Kir4.1 channels which mediate K^+ uptake are crucial for tuning neuronal oscillations, (2) astrocyte depolarisation precedes neuronal oscillation, (3) Genetic silencing of Kir4.1 channels (ShRNAKir4.1) induces a strong locomotor pattern dysfunction. By combining two-photon calcium imaging (gfaABC1D-Gcamp6f, Syn1-jRGECO1a) with electrophysiology and genetic tools (AAVs, ShRNAKir4.1), this project represents a significant breakthrough to (i) better understand how we walk and (ii) broaden the spectrum of therapeutic targets of movement disorders.

Acknowledgement

We are grateful to Dr. Nathalie Rouach (College de France, Paris, France) for her generous gift of transgenic ALDH1L1-eGFP mice. We are grateful to Dr. Frédéric Brocard (INT CNRS UMR7289) for his critical inputs and Anne Duhoux (INT CNRS UMR7289) for animal care. This research was financed by a starting grant from Institut des Neurosciences de la Timone (FI-INT2019 to R.B.).

T10 | Ischemia and hypoxia

T10-001A

The role of IKKb, SRC and RIPK kinases in the adaptation of neuron-glial networks to hypoxia in vitro

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Currently, it has been shown that glial neurotrophic factor (GDNF) and brain neurotrophic factor (BDNF) have a neuroprotective effect in various damages of the nervous system, including hypoxia. However, the described main signaling kinase cascades can explain not all effects exerted by neurotrophic factors. Therefore, the identification of new kinases mediating the action of BDNF and GDNF is an essential task.

The objects of study were primary neuronal cultures obtained from mouse embryos 18 day of gestation (E18). Acute normobaric hypoxia was modeled on day 14 of culture development in vitro. Neurotrophic factors GDNF and BDNF (1 ng/ml) and IKKb, SRC and RIPK kinase inhibitors (1 μM) were added to the culture medium 20 min before hypoxia and in hypoxic medium. The viability of primary nervous cells was estimated on 7th day after hypoxia using the specific fluorescent dyes propidium iodide and bisbenzimide. Calcium events were detected by a specific calcium-sensitive dye Oregon Green 488 BAPTA-1 AM.

Analysis of the viability of primary cortex cultures did not reveal the effect of inhibition of SRC, IKKb and RIPK kinases on cell viability under normal conditions. Hypoxia decreased the number of viable cells in culture (“Sham” 94,34±1,32%, “Hypoxia” 72,33±5,61%). Blockade of SRC and IKKb led to preservation of nervous cell’s viability (90,32±1,2% and 88,33±1,21%), RIPK didn’t change this parameter. BDNF and GDNF maintained cell viability under hypoxic conditions. This neuroprotective effect is retained in the inhibition of studied kinases. Next, we estimated influence RIPK, SRC and IKKb kinases on functional calcium activity of primary neuronal cultures. Hypoxia caused a significant decrease in calcium activity (“Sham” 61,05 ± 0,34%, “Hypoxia” 35,91 ± 1,05% active cell). Blockade of SRC and IKKb kinases did not affect the functional activity of primary hippocampal cultures while blockade of RIPK kinase preserved calcium activity (57.05 ± 11.74%) in post hypoxic period. Application of GDNF and BDNF maintained the functional calcium activity of neuron-glial networks under hypoxic condition (52,94±2,49% and 53,82±1,86%). Our study showed, that inhibition of SRC kinase abolished the action of both GDNF (28.41 ± 1.14%) and BDNF (18.54 ± 3.71%), while the blockade of IKKb and RIPK eliminated the neuroprotective effect of BDNF only.

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T10-002A

Astrocytic volume regulation - contribution of Aquaporin 4 and Transient Receptor Potential Vanilloid 4

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Astrocytes are the main contributors to the maintenance of the brain homeostasis. Their ability to regulate their volume by influx/eflux of osmolytes plays a key role in the brain response to the formation of a cytotoxic edema during ischemic brain injury. In response to the changed extracellular conditions, astrocytes release water and osmolytes via the process termed regulatory volume decrease (RVD). In this study, we aimed to further explore the role of two channels that are suspected to be strongly involved in the process of RVD in astrocytic volume regulation – Aquaporin 4 channels (AQP4) and Transient receptor potential channels, type vanilloid 4 (TRPV4). For this study, we used a subpopulation of EGFP-labelled astrocytes from Aqp4$^{-/-}$, Trpv4$^{-/-}$, Aqp4$^{-/-}$/Trpv4$^{-/-}$ and control (Trpv4$^{+/+}$/Aqp4$^{+/+}$) mice. We induced the astrocytic volume responses in acute cortical slices by multiple ischemia-related stimuli, such as hypoosmotic stress, oxygen glucose deprivation (OGD) and elevated extracellular concentration of potassium cations. We quantified the data using fluorescence intensity-based approach in the whole cells. Afterwards, we examined the levels of expression of specific genes that can contribute to the astrocytic volume regulation in each of our experimental groups using single cell RT- qPCR approach.Based on their volume responses, we identified two distinct subpopulations of astrocytes in controls (further termed high and low responding astrocytes) with different responses to the pathological stimulation and variable incidence between the different pathological stimuli. Moreover, we found similar subpopulation within the astrocytes from Trpv4$^{-/-}$ and Aqp4$^{-/-}$/Trpv4$^{-/-}$ mice. The only exception represents the astrocytes from Aqp4$^{-/-}$ mice exposed to OGD, where we detected only the low type of response. In addition, we were able to detect two distinct subpopulations of astrocytes based on the expression of a specific gene set.

Acknowledgement
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T10-003A

How neonatal macroglia respond to in vitro model of hypoxic ischemic insult?

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Neonatal hypoxia-ischemia (HI) is one of the leading causes of neonatal morbidity and mortality in both developed and developing countries. One of the most fatal consequences of HI is brain injury associated with loss of neurons and glia malfunctioning. Macroglial cells play multiple roles enabling proper functioning of the nervous tissue. Oligodendrocytes create myelin sheath which provides mechanical isolation and allows fast propagation of nerve impulses. Astrocytes are known to metabolically and structurally support neighbouring neural cells. Altogether, the macroglia functions are crucial for keeping local homeostasis, which however can be misbalanced due to HI events

associated with short-time down-regulation of oxygen and trophic supply. Identifying and describing in detail mechanisms initiated after neonatal HI would help to indicate potential targets for therapeutic interventions. Our studies therefore aimed at investigating how a short-term deprivation of glucose and oxygen (OGD) affects selected features of macroglial cells. The primary mixed glial culture was established from 1-2 days old Wistar rats. After 12 days, individual fractions of glial cells were isolated, seeded as monocultures (of oligodendrocyte progenitors or astrocytes, respectively) and then subjected to OGD procedure to mimic HI conditions. After that, the cells were cultured for either 24h or 72h in physiologically normoxic conditions. At the indicated time-points, the immunocytochemical, biochemical and molecular analyses were performed. As revealed by the detailed immunocytochemical examination with a panel of specific antibodies (NG2 and Olig1 for oligodendrocytes; GFAP, EEAT and GS for astrocytes, Ki67 for visualisation of dividing cell nuclei), both types of macroglial cells shortly after the HI significantly increase their proliferation rate. Interestingly, the intensified cell proliferation is not associated with the increase in mitochondria biogenesis as deduced from the calculation of amounts of mitochondrial vs total DNA by means of real-time PCR. The impact of HI insult on neonatal macroglia should be therefore further investigated in the extended study to describe a sequelle of events leading to alteration in withe matter development after neonatal hypoxia-ischemia. The observed statistically significant up-regulation of macroglia proliferation after OGD seems however to be a promising target for strategies aimed at supporting the subsequent cell maturation and their secretory activity.

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T10-004A

Ghrelin receptor agonism protects neurons and astrocytes from excitotoxicity: looking for possible mechanisms-of-action.

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Ghrelin receptor (ghrelin-R) agonism was demonstrated to be neuroprotective and anti-inflammatory in a variety of neurological diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy and ischemia. A prominent common feature in these diseases is excitotoxicity, leading to neuronal loss, inflammation and overall tissue damage. The exact mechanism-of-action or cellular target of ghrelin-R agonism in excitotoxicity remains up to now incompletely understood. Therefore, the aim of this study was to clarify how the ghrelin-R agonist macimorelin affects cell viability, inflammation and autophagy in neurons and glial cells in excitotoxic settings.

SH-SY5Y and mHippoE-14 neuronal cells, 1321N1 astrocytic cells and BV-2 microglial cells were incubated with 0 or 20 mM glutamate, and with concentrations ranging from 0 to 10 μM of the ghrelin-R agonist macimorelin for 24 hours. Afterwards, cell viability, expression of the pro-inflammatory cytokines IL-1β, IL-6, IL-18 and TNF-α, markers of activated signaling pathways and markers for autophagy were assessed.

The ghrelin-R was expressed in all cell types except for BV-2 microglia, which were therefore further omitted from the study. Macimorelin was able to fully rescue glutamate-induced cell loss in astrocytes, and modestly increased cell viability in human and mouse neurons. Macimorelin did not affect pro-inflammatory cytokine expression in basal nor excitotoxic conditions, nor did it affect signaling pathways, except for activation of ERK1/2 in astrocytes in excitotoxic conditions. Lastly, macimorelin exposure blocked autophagy in mouse hippocampal neurons in basal conditions.

Although this study showed that ghrelin-R agonism prevents glutamate-induced cell loss in astrocytes to a higher

extent compared to neurons, the exact mechanisms behind these neuroprotective effects remain unknown. Therefore, further studies are required to determine the molecular processes underlying macimorelin-mediated protection in these cell types in excitotoxicity.

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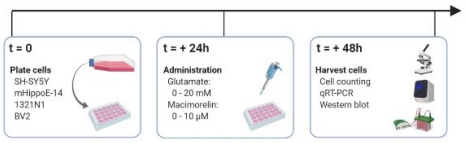

Overview of experimental design.
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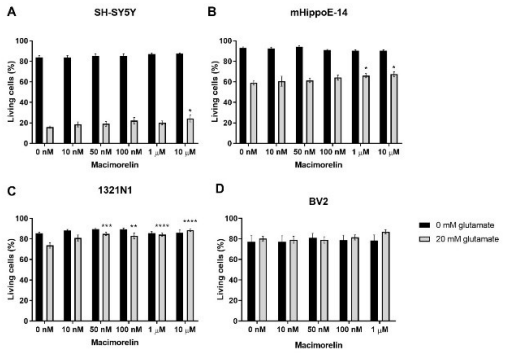

Cell viability in SH-SY5Y, mHippoE-14, 1321N1 and BV2 cells in presence of glutamate or macimorelin.
A) Cell viability in SH-SY5Y cells after glutamate and/or macimorelin administration. B) Cell viability in mHippoE-14 cells after glutamate and/or macimorelin administration. C) Cell viability in 1321N1 cells after glutamate and/or macimorelin administration. D) Glutamate nor macimorelin affected cell viability in BV2 microglial cells. N = 8/16; Two-way ANOVA with Sidak's multiple comparisons test. Significance vs. the 20 mM glutamate - 0 nm macimorelin condition.* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. Data = Mean ± SEM.

T10-005A

Role of the neuropeptide cortistatin in neuro-immune dysregulation and blood-brain barrier dysfunction during ischemic stroke

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Ischemic strokes, the second cause of death worldwide, are the result of the permanent or transient occlusion of a major brain artery. The energy/oxygen deprivation that follows leads to irreversible tissue injury and long-term sequelae. Despite the improvements achieved in strokes management, therapeutic failure is still notorious. For this reason, many studies claim that independent interventions against pathogenic mechanisms should be avoided, while endogenous neuroprotective mediators that globally modulate the immune response should be strengthened. In this context, cortistatin (CST), a neuropeptide widely distributed in the Central Nervous System and the Immune System, that displays anti-inflammatory, immunomodulatory and neuroprotective properties, shows attractive characteristics as a potential endogenous target and as a novel therapeutic agent for strokes. Thus, we are currently studying the involvement of endogenous CST in the immune deregulation and neuroinflammation associated with strokes, as well as its possible therapeutic application. For this purpose, we are using the well-known stroke preclinical model MCAO (middle cerebral artery occlusion) by temporarily occluding (30') the middle cerebral artery in WT and CST-deficient mice ($CST^{-/-}$). Our preliminary results suggest more susceptibility to stroke development and worse prognosis in $CST^{-/-}$ mice 24h post-MCAO, *i.e.* higher neurological score, more extensive neuronal damage and acute glial activation. Moreover, $CST^{-/-}$ mice show a greater blood-brain barrier (BBB) dysfunction after stroke. This BBB breakdown seems to be correlated with a disruption in tight junction proteins (TJ), as shown in a BBB *in vitro* model formed by primary brain endothelial cells isolated from WT and $CST^{-/-}$ mice. There, after oxygen-glucose deprivation, the $CST^{-/-}$ endothelial model shows increased BBB permeability and reduced/altered TJ expression when compared to the WT. Altogether, our results highlight the relevance of endogenous CST modulating neurodegeneration and neuroinflammation episodes during ischemic damage, as well as its key role in the structural and functional viability of the BBB. Accordingly, recovering CST levels by exogenous administration of this neuropeptide may be a novel multifunctional therapeutic agent for the treatment of ischemic stroke.

T10-006A

Does oligodendrocyte progenitor cell's fate go one direction? An impact of neonatal hypoxia – ischemia. What goes right and what goes wrong.

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Neonatal hypoxia-ischemia is one of the main reasons of death of newborn babies. In survived children it often causes the central nervous system hypomyelination, which may result in the long-term neurobehavioral disorders. Myelin, which provides the protection for nerve cell and electrical insulation to facilitate the conduction of impulses through the neuron, is produced by oligodendrocytes. These cells originate from oligodendrocyte progenitors and they start to massively differentiate and mature in the perinatal period. The temporal lack of oxygen and nutrients supply may then contribute to significant changes in the biology of oligodendrocytes for the further development of the brain.

In this study we wanted to evaluate the impact of neonatal hypoxia-ischemia on oligodendrocyte differentiation and myelination. We performed the rat model of asphyxia. It was based on dissection of the left common carotid artery and exposition of P7 animals to 7.5% oxygen for 60 min. In selected timepoints after the injury, we collected brains to perform ELISA analyses of myelin proteins, immunofluorescent visualisation of oligodendrocytes at different stages of maturation and brain myelination.

In the samples of brain tissue collected 4 weeks after the injury, we could detect the increase in the concentration of myelin proteins: PLP, MBP and MAG. Following these results, we analysed coronal brain sections of adult animals (10 weeks after the injury). We could characterize typical macroscopic changes, like the hypoplastic hippocampus, reduced striatum and thinness of corpus callosum. Further immunofluorescent staining with anti-PLP antibody, detecting the main protein of myelin, indicated the reduced myelination in cortex (30% less PLP immunoreactivity, $p<0.001$), striatum (17 % reduction, $p<0.05$) and CA3 of hippocampus (21% reduction, $p<0.05$). Interestingly, when we examined tissues collected 3 days after the injury, we also observed increased proliferation of oligodendrocyte progenitors in the regions of reduced myelination in adults. In main white matter tract, the corpus callosum, we didn't find changes in PLP immunoreactivity. But it turned out, that in brain region we observed the huge decrease in the number of oligodendrocyte progenitor cells (labelled with anti-Olig2) within 3 days after the injury ($p<0.0001$), which was not observed in other analysed areas.

Obtained results may indicate, that hypoxia-ischemia induces serious changes in oligodendrocyte maturation programme. Although it does not inhibit the synthesis of myelin proteins, mature oligodendrocyte seem to fail myelination protocol in several brain areas, corresponding to the outcome observed in babies affected by the injury.

Acknowledgement
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T10-007B

The role of astrocytic p75 neurotrophin receptor on the blood–brain barrier disruption after ischemic stroke.

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The disruption of the blood–brain barrier (BBB) plays a critical role in the pathogenesis of ischemic stroke. It has been reported that p75 neurotrophin receptor (p75NTR) contributes to the disruption of the blood-retinal barrier in retinal ischemia. However, what is the role of p75NTR on the the BBB permeability after acute cerebral ischemia remains unknown. The present study investigated the role and underlying mechanism of p75NTR on the BBB integrity in a middle cerebral artery occlusion stroke model. We report that p75NTR expression was significantly increased in astrocytes and endothelial cells in the infarct area of ischemic mice brain. The BBB leakage after cerebral ischemia was significantly attenuated in genetic p75NTR knockdown (p75NTR+/−) mice. Conditional silencing of astrocytic p75NTR by adeno-associated virus and silencing of p75NTR in astrocytes by siRNA significantly ameliorated BBB disruption in vivo and in vitro, respectively. The mechanisms of astrocytic p75NTR causing BBB disruption involved activation of NF-κB and HIF-1α signals, which upregulated the expression of MMP-9 and vascular endothelial growth factor (VEGF), thus leading to tight junctional protein degradation. Overall, our results provide novel insight into the role of astrocytic p75NTR in BBB disruption after acute cerebral ischemia. p75NTR may therefore be a potential therapeutic target for the treatment of ischemic stroke.

Acknowledgement

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T10-008B

Endo-lysosomal pathway disruption drives microglial phagocytosis dysfunction in stroke

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Microglial phagocytosis of apoptotic cells is an essential process to maintain tissue homeostasis and avoid the spillover of the cytotoxic content that results from the cell death induced by excitotoxicity and/or inflammation. We have shown that microglial phagocytosis is chronically impaired in models of neurodegenerative diseases such as epilepsy and stroke, where microglial phagocytosis is blocked as early as 6 hours after transient Medial Cerebral Artery occlusion (tMCAo), a model of stroke. Here, we hypothesize that microglial phagocytosis impairment in tMCAO was related to an energetic failure and used different in vitro systems to test the role of oxygen and nutrient deprivation (OND). We first treated primary microglial cultures with OND and observed phagocytosis deficits, in particular, a reduced degradation of apoptotic cells. This reduction was related to an increased lysosomal pH, possibly as a consequence of alterations in energy-dependent proton pumps that lead to a deficient enzymatic activity. The energetic dysfunction not only affected phagocytosis but also autophagy, another endosomal pathway that converges in the lysosome. We assessed autophagy using electron microscopy and found an increase in autophagy-like vesicles, presumably due to a stallment of the autophagosomes related to a deficient lysosomal

degradation. To confirm the effect of OND on phagocytosis we next used hippocampal organotypic cultures and observed a similar defect in apoptotic cell phagocytosis, which was related to a reduced microglial surveillance and process motility by 2-photon microscopy, likely related to the generalized energetic failure that takes place in stroke. The OND-induced phagocytosis reduction was rapidly recovered after reperfusion suggesting that, in addition to the acute energetic failure, a more complex mechanism is responsible for the long-term impairment of phagocytosis found in tMCAo mice. In order to revert the phagocytosis impairment, we tested the autophagy inductor rapamycin, both in vitro and in vivo, to restore the autophagy flux and the altered endo-lysosomal pathway, and hence, recover the phagocytic activity. Thus, the microglial phagocytic potential opens a novel approach to accelerate the recovery of the ischemic brain by harnessing microglial phagocytosis of apoptotic cells through the stimulation of the autophagy.

T10-009B

Subventricular zone gliogenesis after stroke

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Cell regeneration in adult mammalian brain can occur in the subventricular zone (SVZ) after an insult in terms of neurogenesis or gliogenesis. We previously demonstrated that oxygen and glucose deprivation, both in vitro and in vivo by MCAO, stimulated the SVZ generation of newborn fibrotic astrocytes expressing high levels of the protein Thbs4. In this study, we characterized by immunofluorescence the activation of astrogliogenesis after brain ischemia and newborn astrocytes distribution within the affected brain areas. After 15-30 days post ischemia, we observed brain damage in the cortex and striatum. Thbs4^{+} astrocytes migrated preferentially to the ipsilateral cortex whereas migration to the olfactory bulb was reduced. Using serial cuts of an organotypic cultures from SVZ-cortex-striatum we could reconstruct the topography of the SVZ activated Thbs4^{+} astrocytes and identify the subregional localization of astrocytes progenitor cells. Oxygen and glucose deprivation in organotypic cultures induced antero-dorsal and postero-medial SVZ population activation. In addition to ex vivo studies, in vivo post-natal electroporation was carried out to distinguish among NSC activation. Further experiments are needed to elucidate the role of ischemia-induced astrogliogenesis in adult brain.

T10-010B

The Renin-Angiotensin-Aldosteron System modulates astrocytes and their crosstalk with microglia and neurons

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Astrocytes are involved as the most abundant cell type of the central nervous system (CNS) in homeostasis, neuroprotection, and regulation of synaptic plasticity. They have emerged as modulating factors in various neurological diseases such as cerebral ischemia. One of the leading causes of stroke is high blood pressure, which is effectively regulated by the Renin-Angiotensin-Aldosteron System (RAAS). However, the direct effect of the RAAS on astrocytes and their consecutive interaction with other cell entities of the CNS is still unknown.
The current study aimed to elucidate the effects of angiotensin II and its receptor blockers telmisartan (angiotensin II type 1 receptor-blocker) and PD123319 (angiotensin II type 2 receptor-blocker) on both resting and reactivated astrocytes. Furthermore, the conditioned medium (CM) of these astrocytes was used to investigate the interaction with microglia and cortical neurons, respectively. Oxygen- and glucose deprivation (OGD) served as a translational model of ischemic stroke *in vitro*. Microelectrode Arrays (MEAs) were used to quantify neuronal activity *in vitro*.
Telmisartan increased cell survival of astrocytes during acute as well as prolonged OGD conditions. PD123319 affected the activation phenotype by increasing A2-marker expression. Proliferation activity was unchanged by telmisartan, but PD123319 promoted proliferation of resting astrocytes.
Increased neuronal activity was observed after treatment of neurons with CM of Telmisartan stimulated astrocytes. Furthermore, the CM of PD123319-stimulated astrocytes increased the expression of pro-inflammatory markers in microglia while reducing anti-inflammatory markers.
Data suggest that RAAS and its inhibitors affect the phenotype of differentially activated astrocytes and their interaction with neurons and microglia. Positive CNS-effects of telmisartan may be exploited in the clinical situation of stroke. Further mechanistic understanding could potentially serve as a promising targeted therapy in the chronic stage of cerebral ischemia.

T10-012B

Exploring differential sensitivity of ischemia at the tripartite synapse with a mathematical model

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Neuronal communication critically depends on the well-functioning of neurons and astrocytes at the tripartite synapse. In low energy conditions, the primary consumer of energy, the Na^+/K^+-ATPase (NKA) fails, which triggers a cascade of pathological events caused by a breakdown of ion gradients, such as membrane depolarization, cell swelling, and excitotoxicity. These are typical early events that occur during an ischemic stroke. The severity of these pathologies depends on various factors including age and brain region, but the reasons for this differential sensitivity are far from understood. In this work, we explore the differential behavior of synaptic recovery in low-energy conditions, using a mathematical model (see Fig. 1). The model is built from biophysical principles and describes the temporal dynamics of Na^+, K^+, Cl^-, Ca^{2+} and glutamate by explicitly modeling relevant ion transport

behavior. The dynamics are described in neuronal, astrocyte, and extracellular compartments, and are calibrated to experimental data. By varying strength and duration of ischemia, we observe two stable resting states in response to short-term ischemia. These correspond to baseline and pathological resting states. We then vary baseline extracellular volume fraction and NKA pumping strength to analyze the sensitivity of these resting states. The model predicts that the transition to the pathological state is favored if the extracellular volume fraction is small (see Fig. 2) and if the baseline NKA pumping strength is weak. These variances are present in differing brain regions and ages, thereby providing translational relevance for understanding the differential vulnerability of ischemic stroke. Moreover, the model predicts that blocking neuronal voltage-gated Na^+ and K^+ channels assists in synaptic recovery from the pathological state, providing insight for possible therapeutic targets. We also present an easy-to-use graphical user interface (GUI) to simulate our model, which can be used to compare with relevant experimental data.

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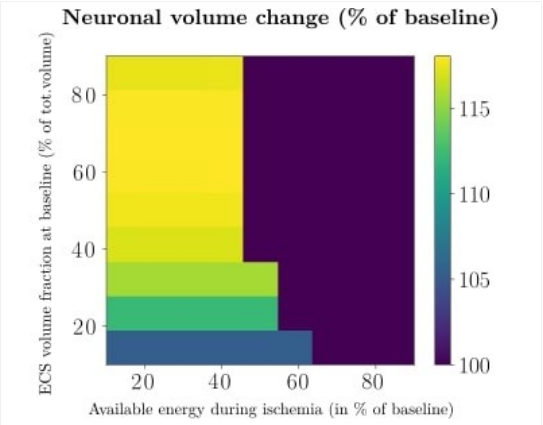

Figure 2: Neuronal volume change as a function of ECS volume fraction and extremity of ischemia.

Result

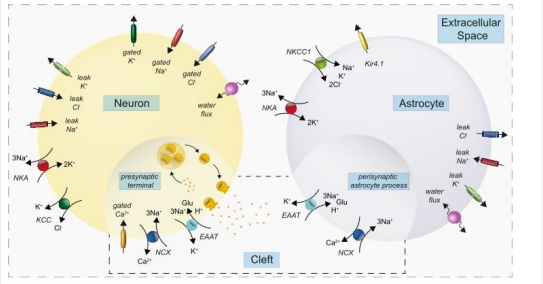

Figure 1: Scheme of the mathematical model. We show the various ion channels and cotransporters that are explicitly modeled to produce dynamics of Na^+, K^+, Cl^-, Ca^{2+} and glutamate.

Model scheme

T11 | Memory and learning

T11-001E

Serotonergic signaling in microglia is critical for spatial memory consolidation

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The hippocampus receives dense innervation by serotonergic projections from dorsal raphe nuclei and several studies demonstrate that serotonin (5-HT) modulates hippocampal plasticity and memory formation. We previously showed that, besides neurons, microglia are able to sense and respond to 5-HT via 5-HT_{2B} receptors, the main 5-HT receptors expressed by microglia[1]. Microglia are dynamic cells that constantly survey the environment by physically interacting with neurons and shaping synaptic connections. In addition to their well-established impact on postnatal brain maturation, several studies unveiled a major role for microglia in maintaining brain homeostasis during adulthood, modulating neuronal activity and shaping synaptic and structural plasticity[2-8]. In line with these data, microglial depletion or deletion of a number of microglial genes affects learning, memory, and forgetting[2-3,5,6,8]. In this study, we investigated whether disturbed communication between microglia and neurons due to conditional invalidation of 5-HT_{2B} receptors in microglia in adult mice affects hippocampal-dependent memory processes. We demonstrate that absence of microglial 5-HT_{2B} receptors disrupts long-term spatial memory. This deficit is not due to impaired memory encoding or changes in sleep architecture, but is associated with reduced learning-induced spine remodeling in the hippocampus, suggesting a defect in memory consolidation in the absence of 5-HT signaling in adult microglia. Moreover, we unraveled an abnormal morphological phenotype of microglia in the absence of 5-HT signaling, together with a potentiation of excitatory transmission normally observed in response to high frequency stimulations at CA3-to-CA1 synapses. Altogether, these findings suggest that microglia deficient in 5-HT_{2B} receptors may lose their ability to interact with neuronal elements, which subsequently dysregulates plasticity and memory.

Acknowledgement

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T11-002E

Metaplastic switching by astrocytes

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An experience has the potential to become either a long-lasting memory, or a faded matter from the past. In other words, the same input can be altered into different outcomes of memory. The difference in the brains operative state is most likely an alternating factor that would have an effect on memory formation due to neuronal metaplastic changes. Astrocytes are known to play a significant role in regulating the brain's environment, and recently considered as a key component in the memory formation process. We suggest that astrocytic activity modulates neuronal metaplastic states, acting as a switch between different circumstances. To assess the astrocytic contribution to memory, a fear conditioning paradigm was conducted on transgenic mice. The mice were conditioned to associate an electrical foot-shock to a 10 kHz tone cue and the contextual conditions during the shock. The degree of memory formation was analyzed from freezing behaviors when exposed to the cue or the context. Astrocyte specific optogenetic stimuli in the amygdala was applied during conditioning, and the effects on fear memory formation was studied. Glial specific ChR2 activation resulted in an increase of freezing during conditioning, but contrary to expectations, fear memory formation was suppressed. This may be the result of the disruption of neural encoding in the consolidation process. In contrast, glial specific ArchT activation had no effect on the freezing behavior directly after the foot-shock, but fear memory formation seemed to be augmented. Enhanced memory consolidation was likely triggered by the astrocytic ArchT activation. Activation of ChR2 induces proton influx, as well as calcium and sodium influx. On the other hand, ArchT activation results in proton extrusion. Therefore, changes in intra-astrocytic pH could be the definitive factor of metaplastic modulations, switching between memorable and forgetful events. In order to determine the time-dependent influences of such ionic dynamics in astrocytes, and its relevant role during memory formation, measurements in freely moving mice, utilizing the fiber photometry method, were conducted. In our lab, we have developed the methodology for real time fluorescent imaging of intra-astrocytic ion concentrations, such as pH and calcium, via the transgenic expressions of molecular sensors.

T11-003E

The Role of Astrocytes in Spatial Cognition

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In recent years, groundbreaking research revealed many surprising roles for astrocytes in modulating neuronal activity and even behavior. While astrocytes in cortical areas were shown to respond to specific sensory stimuli, their activity in awake mice performing a multisensory cognitive task has not been studied. To investigate the

functional role of astrocytes, we used 2-photon calcium imaging in dorsal CA1 of mice running in a virtual reality apparatus. We imaged dozens of astrocytes as mice ran in a familiar circular virtual maze, in which a reward was given in a constant location, and examined whether astrocytes independently encode spatial and non-spatial features of the environment. Astrocytes did not have bell-shaped tuning curves, typical to neuronal place cells, but rather appeared to increase their activity as they approached the previously learnt reward location. Using machine learning, we were able to successfully decode the mouse location from the astrocyte activity alone. We next imaged the same population of astrocytes while the mice were introduced to a novel virtual environment differing in visual and tactile cues. Notably, the astrocytic activity was no longer modulated by the location, suggesting that the elevation of activity towards a rewarding location requires familiarity with the environment. Our results shed light on the computational capabilities of astrocytes, their role in learning a new environment until it becomes familiar, and their contribution to cognitive functions.

T11-004E

Dysregulation of astroglial cAMP signals and glucose utilisation in an intellectual disability *Gdi1*-null mouse model

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X-linked intellectual disability (XLID), clinically characterized by “pure” cognitive deficit without additional clinical features, is caused by mutations in the human *GDI1* gene encoding for αGDI, which controls the cycling of small GTPases involved in regulation of vesicle trafficking. *Gdi1* knockout (KO) and conditional *Gdi1* KO restricted to neurons in the anterior forebrain, impairs working and associative short-term memory in mice. Not only neurons, but also astrocytes, a type of homeostasis providing neuroglial cells, are involved in the regulation of memory formation and consolidation. Astrocyte-derived L-lactate, generated through aerobic glycolysis, is transported to neurons to support memory formation and consolidation through cAMP-dependent process (1). L-Lactate can also act as an astroglial signal, activating intracellular cAMP signals, and enhancing astroglial aerobic glycolysis and L-lactate production (2), providing a positive feedback mechanism for L-lactate production that may sustain L-lactate gradient from astrocytes to neurons required for memory formation. Whether extracellular L-lactate-mediated cAMP signalling and glucose metabolism are altered in astrocytes isolated from *Gdi1* KO animals is poorly understood.
We performed real-time measurements of cytosolic cAMP (indirectly via cAMP-dependent PKA activity), glucose, and lactate using Förster resonance energy transfer (FRET) microscopy in single cultured astrocytes from *Gdi1* wild-type (WT) and *Gdi1* KO animals. When cells were exposed to extracellular L-lactate (2 mM), the increase in cAMP was observed only in *Gdi1* KO, but not in *Gdi1* WT astrocytes, while 20 mM L-lactate increased cAMP in both *Gdi1* WT andKO cells. Noradrenaline (100 μM) increased cAMP production in both *Gdi1* WT and KO astrocytes, but the cAMP production was slower in *Gdi1* KO astrocytes. Pre-treatment of cells with 2 mM L-lactate facilitated noradrenaline-mediated cAMP production in *Gdi1* KO cells, but not in *Gdi1* WT cells, suggesting

increased sensitivity to extracellular L-lactate in *Gdi1* KO cells. We observed decreased free glucose mobilisation in *Gdi1* KO astrocytes upon L-lactate (20 mM) and noradrenaline (200 µM) stimulation, respectively, compared to *Gdi1* WT astrocytes. This may suggest decreased free glucose permeability through membrane glucose transporters and/or facilitated free glucose utilisation. Receptor-augmented aerobic glycolysis (*i.e.,* L-lactate production) was, however, unaltered (3).

Our results revealed increased astroglial sensitivity to extracellular L-lactate in *GDI1* gene-associated XLID, likely through an unknown L-lactate receptor stimulating cAMP signalling in this neurodevelopmental disorder.

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T11-005E

Memory formation upon food deprivation: glia fuel neurons with locally synthesized ketone bodies

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Food scarcity represents a major metabolic challenge for the brain and processes that ensure its adequate functioning are considered crucial for organism survival. The brain is the central regulator of energy homeostasis, and it gives priority to its own supply over peripheral organs. However, the mechanisms through which the energy status regulates brain plasticity remain largely unknown. We have previously revealed using *Drosophila melanogaster* that the brain is subjected to adaptive plasticity and that under a metabolic challenge, such as starvation (i.e when glucose levels scarce), the brain disables the costly formation of protein synthesis dependent long-term memory (LTM) to favor survival (1). Recently, we have demonstrated that flies double their sugar intake and upregulate their mitochondrial pyruvate flux in the fly olfactory memory center, the Mushroom Body (MB), after LTM formation (2). Nevertheless, in absence of glucose derivatives, how does the brain regulate memory formation to withstands periods of food scarcity? And, what are the metabolic fuels and cell types used by the brain to sustain the formation of memory upon starvation?

Ketone Bodies (KBs) are energy rich metabolites, derived from fatty acid metabolism and used by the brain under starvation. However, it is currently unknown whether neurons are able to use KBs to sustain neuronal physiology and in particular memory formation. Here, we addressed the *in vivo* role of KBs metabolism in aversive olfactory memory formation upon starvation, the metabolic pathways required and the cell types involved in this process by combining genetics, behavioral assays, lipid droplets staining and *in vivo* two-photon imaging.

By using cell type specific gene knockdown restricted to adulthood associated to behavioral and *in vivo* imaging experiments, we found that KBs import and mitochondrial oxidation are required in MB neurons to sustain memory formation upon starvation. Furthermore, we identified cortex glial cells, a perisomatic glial subtype, as the ones providing KBs to neurons for memory formation. Eventually using behavioral assays and lipid staining, we demonstrated that memory formation upon starvation is dependent on ketogenesis in cortex glia from their own

lipid droplets stores and that this process is likely regulated by the master energy sensor AMPK.

In conclusion, here we propose a model of cortex glia-MB neurons interactions specific to starvation in which KBs synthetized by glia from their own lipid droplets stores are transported to neurons to sustain memory formation upon starvation (Figure model). Our data suggest that, at least in glial cells, AMPK could be a key molecular switch to activate this mechanism.

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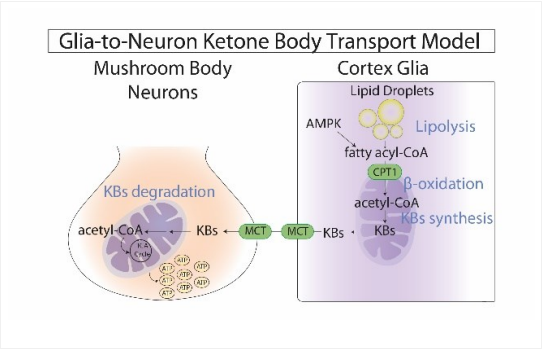

Figure model

T11-006E

Memory consolidation is governed by gap junctional communication in the astrocytic network

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There is a growing body of evidence for the involvement of astrocytes in oscillatory brain activity, both in physiological (e.g. slow wave activity) and pathophysiological processes (e.g. epilepsy). By exploring various molecular interactions between neuronal and astrocyte networks, we have previously shown that blocking astrocytic gap junctions suppresses slow wave activity in rats (Szabó et al. 2017) and also inhibits epileptiform activity in acute hippocampal slices (Vincze et al., 2019), suggesting a causal role of astrocytes in neuronal synchronizations.

Since slow wave sleep is associated with memory consolidation, perturbation of the astrocytic syncytium during this process may impact the working memory of rats. To this end, we activated astrocytic gap junctions using trimethylamine (TMA), or inhibited them with an astrocyte-specific connexin 43 (Cx43) antibody. Memory consolidation was evaluated using novel object recognition tests. Our results show that opening of gap junctions by TMA significantly enhances memory formation (Fig. 1B), while inhibition by Cx43 antibody impairs memory performance (Fig. 2B). Furthermore, we have found that TMA seems to have a greater effect on animals with

weaker baseline performance (Fig. 1A).

In addition to slow wave activity, we are also investigating the impact of astrocytes on pathophysiological oscillatory activity, specifically epileptic seizure-like events. We explored the effects of TMA and the Cx54 antibody on the calcium signalling of neurons and astrocytes using two-photon microscopy in genetically epileptic WAG/Rij rats.We conclude that large-scale synchronization in the astrocyte network through gap junctions plays a previously unrecognized, essential role in higher cognitive functions and may open up new avenues in the therapy of cognitive disorders.

Acknowledgement

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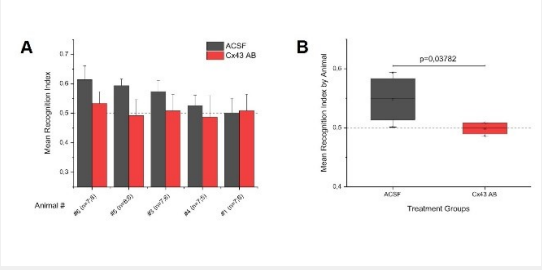

Fig. 2. The Cx43 antibody decreases memory performance

*(**A**)Blockade of gap junctions with a Cx43 antibody (red columns) decreased the mean recognition index of rats compared to control treatments with ACSF (grey columns).(**B**) Comparing the mean recognition indices of all animals between treatment groups shows that the Cx43 antibody significantly decreases memory performance (p=0.038).*

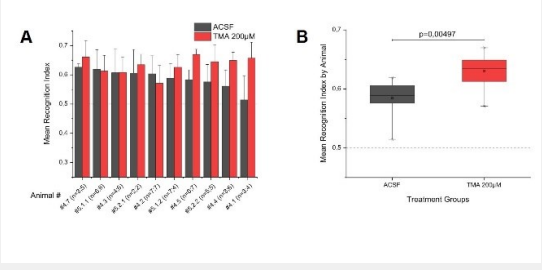

Fig. 1. TMA increases memory performance

*(**A**)Opening of gap junctions with trimethylamine (TMA, red columns) improved the mean recognition index of individual rats showing weaker memory performance in control experiments (grey columns).(**B**) Comparing the mean recognition indices of all animals between treatment groups shows that TMA significantly increases memory performance (p=0.005).*

T11-007E

Microglia and Complement Signaling Contribute to Hippocampal Spatial Tuning

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Microglia, the resident immune cells in the brain, survey their local environment and sense neuronal activity. They have been shown to regulate activity-dependent synaptic plasticity, a process important for learning and memory. The complement cascade is one molecular pathway involved in microglia-mediated synaptic pruning, and complement-associated synapse loss has been implicated in memory impairment. The hippocampus is well known for its role in spatial memory and contains place cells, or neurons that fire preferentially to specific locations within an environment. While microglia and related factors contribute to hippocampal memory and plasticity, the corresponding effects on network activity are still being elucidated. By utilizing longitudinal in vivo imaging of hippocampal calcium traces, we investigate the effects of microglia and complement on spatial tuning of hippocampal neuronal activity, as well as on hippocampal-dependent behavior. First, we study the physiological role of microglia by depleting them from the brain. Second, we knock out complement component C1q in microglia to determine the contribution of this pathway to hippocampal function. Finally, using an inflammatory model we investigate how spatial tuning may be affected in pathogenic conditions. Preliminary data suggest that microglia ablation or loss of microglial complement signaling alters hippocampal activity and impairs spatial tuning. Longitudinal analysis of individual neuron activity will elucidate whether microglia-mediated remodeling is involved in hippocampal remapping across days. These experiments will further our understanding of how microglia can impact hippocampal function on a network level.

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T12 | Myelin

T12-001A

How do astrocytes influence myelination?

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In recent years, the active role that astrocytes play in brain development has been brought to the forefront by seminal studies. However, many fundamental characteristics and functions of astrocytes remain to be uncovered. The contribution of astrocytes to myelin maintenance and re/myelination, affected in a large number of pathological conditions of the CNS, remains as one of these mysteries. To answer these questions, we identified a large number of bioactive proteins and lipids in the astrocytic secretome and study their importance during the myelination process. Primary immunopurified rat retinal ganglion cells (RGC) and cortical oligodendrocytes (OPC) are co-cultured *in vitro* and treated with processed and fractionated astrocyte conditioned media (ACM) or single factors. High content imaging of immunofluorescent stained co-cultures and our novel computer-vision algorithm permit an automated and unbiased quantification of myelination. We show that astrocytes produce factors supporting neurons and oligodendrocytes during the myelination process and unravel the secreted Protein X as an enhancer of late-stage myelination in co-cultures *in vitro* and *ex vivo* brain slices. Significant downregulation of Protein X by reactive astrocytes proposes it as a potential neuroprotective factor. Furthermore, developmental myelin formation of Protein X KO mice does not differ from WT mice indicating that Protein X is necessary but not sufficient for developmental myelination *in vivo*. Together, we have found a new relevant factor that assists the myelination process and might be a clinically relevant target for the improvement of the treatment of Multiple Sclerosis.

T12-002A

Lanthionine ketimine ethyl ester increases remyelination in the cuprizone model of demyelination

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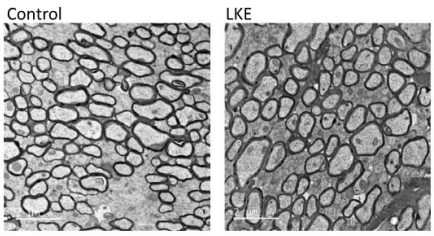
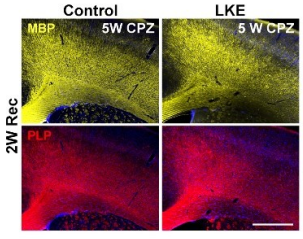
Lanthionine ketimine ethyl ester (LKE) is a derivative of the naturally occurring amino acid lanthionine. We previously showed that LKE increases oligodendrocyte (OLG) progenitor cell (OPC) differentiation in vitro and reduced clinical signs in the MOG peptide EAE mouse model of multiple sclerosis (MS). To test if LKE increases remyelination, we induced demyelination by feeding cuprizone (CPZ) diet to C57Bl6 mice. To assess effects on remyelination, after 5 weeks of CPZ diet, mice were placed onto normal diet, or normal diet containing 100 ppm LKE for two weeks, then brains were examined for indices of remyelination. Electron microscopy (EM) was used to measure axon caliber and myelin thickness in sections prepared from the corpus callosum, and immunohistochemistry (IHC) used to quantify changes in markers of OLG differentiation and astrocyte activation. IHC confirmed that CPZ reduced the numbers of Olig2+ and CC1+ OLGs and myelin basic protein (MBP) and proteolipid protein (PLP) staining. Significant increases in proliferating (Ki67+) OPCs and in GFAP+ astrocyte activation was also observed. After 2 weeks post-CPZ, brains from mice provided the LKE-containing diet had a significant increase in the number of Olig2+ and CC1+ OLGs and MBP/PLP protein staining as compared to mice provided the normal diet. However, no change in the numbers of proliferating OPCs or GFAP+ astrocyte activation was observed. In addition, ultrastructural EM analysis showed that remyelination in the LKE-treated mice had significant increases in average myelin thickness, without effect on average axon diameter, leading to an overall increase in average G-ratio. These results demonstrate that LKE increases the degree of remyelination and suggest this may be due to an increase in OLG differentiation and OPC/OLG cell survival.



LKE increases myelin thickness
After 2 weeks of remyelination, mice provided LKE chow have thicker myelin in corpus callosum than mice given control chow



LKE increases MBP and PLP staining
After 2 weeks, mice provided LKE chow have increased MBP and PLP staining in the corpus callosum compared to mice given control chow

T12-003A

Activity-dependent nanoscale dynamics of myelinated axons

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Neural firing has long been suspected to accompany nanometer-scale mechanical deformations, which were experimentally shown in large invertebrate axons1 and cultured neurons in vitro2. Although the saltatory conduction in myelinated axons has exquisite sensitivity to periaxonal cytoarchitecture, it is still veiled whether there exist any nanostructural dynamics of myelinated axons during the propagation of action potentials3. To this end, here we introduced a novel all-optical neurophysiology approach to a living brain tissue, which triggers axonal firing by optogenetics and simultaneously records nanostructural dynamics by spectral reflectometry (SpeRe)4. First, we optimized the SpeRe system to provide the millisecond-scale temporal resolution as well as subnanometer-scale spatial precision. The system performance was verified using a tapered glass fiber and a liquid crystal microsphere under a time-varying electric field. Second, we performed theoretical simulation to identify a spectral feature representing the nanostructural dynamics of the periaxonal space, and found that the hypsochromic spectral shift is a robust marker for the nanoscale dynamics. Finally, by applying optogenetic stimulation on excitatory neurons in a brain slice, we revealed nanostructural dynamics of myelinated axons, which was in sync with optogenetic stimuli and was abolished when treated with a sodium channel blocker. By providing unprecedented information on activity-dependent dynamics of myelinated axons, our study will contribute to unveil the underscored functional role of axo-myelinic structural dynamics in neural circuit function.

Acknowledgement
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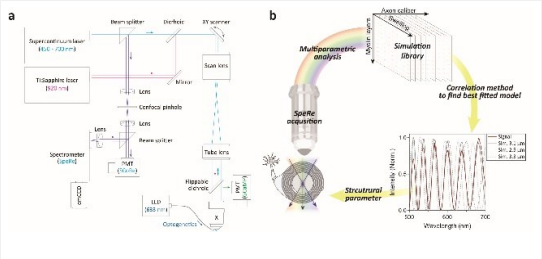

Optic setup and concept of SpeRe (Spectral Reflectometry)
a, Optic diagram of SpeRe. Combined two optical imaging system with Two-photon and SpeRe. Stimulation light for optogenetics delivered by the optical fiber to the brain tissue surface at an oblique. **b,** Pipeline for SpeRe signal acquisition and obtaining structural information.

T12-004A

CMTM6 expressed on the adaxonal Schwann cell surface restricts axonal diameters in peripheral nerves

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Myelination of axons accelerates nerve impulse propagation 20-100-fold, theoretically allowing rapid nerve conduction with reduced axonal diameters. However, no myelin dependent signal has been described to restrict axonal diameters. Hypothesizing that numerous proteins that mediate cross-talk between axons and Schwann cells remain to be discovered, we assessed a biochemical fraction enriched for the axon/myelin-interface by label-free proteomics. By STED-microscopy, immunoblotting and cryo-immuno electron microscopy we confirm the localization of the novel peripheral myelin protein CMTM6 (chemokine-like factor-like MARVEL-transmembrane domain-containing protein 6) at the adaxonal Schwann cell membrane. Genetic disruption of *Cmtm6* expression in Schwann cells causes a substantial increase of axonal diameters in various peripheral nerves without impairing myelin biogenesis or axonal integrity. Diameters of non-myelinated axons are also increased when CMTM6 is lacking from Schwann cells. Importantly, radial sorting of axons and myelin biogenesis are not compromised. Increased axonal diameters correlate with accelerated sensory nerve conduction velocity, enhanced sensory responses and perturbed motor performance. We further demonstrate that loss-of-CMTM6-function causative of larger axonal diameters overrides loss-of-MAG-function, which by itself causes a shift towards reduced axonal

diameters. Together, Schwann cells utilize adaxonal proteins including MAG and CMTM6 to regulate radial axonal growth and optimize nerve function. In transgenic gain-of-function studies we currently assess the relevance of CMTM6 in regulating the age-dependent increase of axonal diameters in peripheral nerves.

T12-005A

Neuron class–specific responses govern adaptive myelin remodeling in the neocortex

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Myelin plasticity is critical for neurological function, including learning and memory. However, it is unknown whether this plasticity reflects uniform changes across all neuronal subtypes, or whether myelin dynamics vary between neuronal classes to enable fine-tuning of adaptive circuit responses. We performed *in vivo* two-photon imaging of myelin sheaths along single axons of excitatory callosal neurons and inhibitory parvalbumin-expressing interneurons in adult mouse visual cortex. We found that both neuron types show homeostatic myelin remodeling under normal vision. However, monocular deprivation results in adaptive myelin remodeling only in parvalbumin-expressing interneurons. An initial increase in elongation of myelin segments is followed by contraction of a separate cohort of segments. Sensory experience does not alter the generation rate of new myelinating-oligodendrocytes, but it displays the capacity to recruit pre-existing oligodendrocytes to generate new myelin sheaths on PV^+ interneurons. An increase in axonal branch remodeling is revealed along with this enhancement of myelin plasticity. This data[1] indicates that distinct classes of neurons individualize remodeling of their myelination profiles to diversify circuit tuning in response to sensory experience.

Acknowledgement

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T12-006A

Organization of ion channel domains in myelinated inhibitory neurons of the hippocampus

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Recent reports indicate that GABAergic neurons, in particular the parvalbumin (PV) interneurons, are frequently myelinated[1,2]. Fast-spiking PV neurons are highly energy-demanding cells for which myelin may provide axonal metabolic support in addition to optimizing action potential propagation. Myelination is discontinuous along the PV ramified axons and the mechanisms controlling myelin pattern as well as the segregation of ion channels at the node of Ranvier and heminodes require investigations. We showed recently that the PV interneurons are highly enriched in Kv1 channels associated with the juxtaparanodal molecules TAG-1/Contactin2, Caspr2, ADAM22, and 4.1B all along the axon before myelination[3]. The Kv1.2 channels are then clustered at the juxtaparanodes or hemi-juxtaparanodes in PV myelinated axons of the adult hippocampus. We analyzed the distribution of panNav at nodes, heminodes, and branch points in Lhx6+ and PV+ myelinated axons of the CA1 hippocampus. The nodal Nav channels are extending up to a 9 µm distance between two paranodes. Heminodal clustering of Nav channels is observed for more interspaced paranodes. At the age of P30, 44% of the nodes display a length>2 µm and only 22 % at P70, indicating that the internodes may still elongate during this period of maturation. We characterized mouse genetic models with alterations of myelinated inhibitory axons. The PV-Cre; ADAM23$^{LoxP/LoxP}$ mice display severe alteration (-78%) of the Kv1 juxtaparanodal clustering as analyzed at P70. Thus, ADAM23 acts as a novel member of the Kv1 complex in the CNS, in addition to Caspr2 and Contactin2. Next, we analyzed the phenotype of 4.1B KO mice in the hippocampus and observed a selective loss of myelin in the stratum radiatum of the CA1 and CA3 regions both at P30 and P70. Strikingly, myelination seems to be interrupted in the PV axons crossing the stratum radiatum (see the Figure). Moreover, the Kv1 juxtaparanodal clustering is severely affected (-54%), and the heminodal Nav channels mislocalized in 4.1B-deficient mice. We plan now to analyze the physiological properties of PV cells in these genetic models to provide insights into the function of myelin in this type of interneurons.

Acknowledgement

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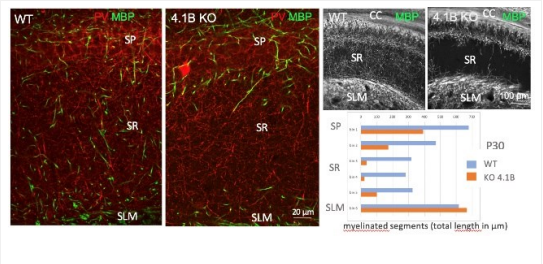

Myelination is severely altered in the hippocampus of the 4.1B KO mice
Immunostaining for parvalbumin PV (red) inhibitory neurons and MBP (green). Wild-type and 4.1B KO mice at P30 in the CA1 region of the hippocampus. Note the almost complete loss of myelinated axons in the stratum radiatum (SR) in 4.1B KO mice although myelination is preserved in the corpus callosum (CC) and stratum lacunosum-moleculare (SLM). Graph showing thelength of myelinated segments measured in the CA1 region divided in 6 bins (area 300x40 µm) from the stratum pyramidale (SP) to the SLM.

T12-007A

Hypoxia-inducible factor 1 alpha promotes peripheral nerve myelination.

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Schwann cells (SCs) generate myelin sheath in the peripheral nervous system (PNS), and their differentiation plays an important role in PNS myelination. Mature SCs are formed via a stepwise differentiation during development from neural crest cell-derived SC precursors to immature SCs and pro-myelinating SCs, followed by myelinating/non-myelinating SCs. Characteristic expression of genes, including several transcriptional factors, have been described in each developmental stage. However, underlying mechanisms of SC differentiation remain unclear. Recent studies have shown that hypoxia-inducible factor-1 alpha (HIF-1α) plays a role in myelination in the central nervous system. Here, we investigated the role of HIF-1α in PNS myelination. We found that HIF-1α can be stabilized in nuclei of SCs cultured under hypoxia (1% O2). Culturing in hypoxia or overexpression of HIF-1α bearing mutation to be resistant to proteasomal degradation resulted in upregulation of myelin related gene expressions in SCs. By immunohistochemistry, HIF-1α was localized in S100β-positive SCs in murine sciatic nerve during development. HIF-1α expression in protein level was higher during development than in adulthood in mice, while HIF-1α mRNA expression was almost constant. Moreover, HIF-1α stabilizing drug that inhibits prolyl hydroxylation was able to upregulate myelin protein expression and promoted myelination in culture. Transient hypoxic incubation also facilitated in vitro myelination. These finding suggest that HIF-1α induces SCs differentiation and promotes PNS myelination.

T12-008A

Myelin biogenesis is associated with pathological ultrastructure that is resolved by microglia during development

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To enable rapid propagation of action potentials, axons are ensheathed by myelin, a multilayered insulating membrane formed by oligodendrocytes. Most of the myelin is generated early in development, in a process thought to be error-free, resulting in the generation of long-lasting stable membrane structures. Here, we explored structural and dynamic changes in CNS myelin during development by combining ultrastructural analysis of mouse optic

nerves by serial block face scanning electron microscopy and confocal time-lapse imaging in the zebrafish spinal cord. We found that myelin undergoes extensive ultrastructural changes during early postnatal development. Myelin degeneration profiles were engulfed and phagocytosed by microglia in a phosphatidylserine-dependent manner. In contrast, retractions of entire myelin sheaths occurred independently of microglia and involved uptake of myelin by the oligodendrocyte itself. Our findings show that the generation of myelin early in development is an inaccurate process associated with aberrant ultrastructural features that requires substantial refinement.

T12-009A

Microglia facilitate repair of demyelinated lesions via post-squalene sterol synthesis

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The repair of inflamed, demyelinated lesions as in multiple sclerosis (MS) necessitates the clearance of cholesterol-rich myelin debris by microglia/macrophages and the switch from a pro-inflammatory to an anti-inflammatory lesion environment. Subsequently, oligodendrocytes increase cholesterol levels as a prerequisite for synthesizing new myelin membranes. We hypothesized that lesion resolution is regulated by the fate of cholesterol from damaged myelin and oligodendroglial sterol synthesis. By integrating gene expression profiling, genetics and comprehensive phenotyping, we found that, paradoxically, sterol synthesis in myelin-phagocytosing microglia/macrophages determines the repair of acutely demyelinated lesions. Rather than producing cholesterol, microglia/macrophages synthesized desmosterol, the immediate cholesterol precursor. Desmosterol activated liver X receptor (LXR) signaling to resolve inflammation, creating a permissive environment for oligodendrocyte differentiation. Moreover, LXR target gene products facilitated the efflux of lipid and cholesterol from lipid-laden microglia/macrophages to support remyelination by oligodendrocytes. Consequently, pharmacological stimulation of sterol synthesis boosted the repair of demyelinated lesions, suggesting novel therapeutic strategies for myelin repair in MS.

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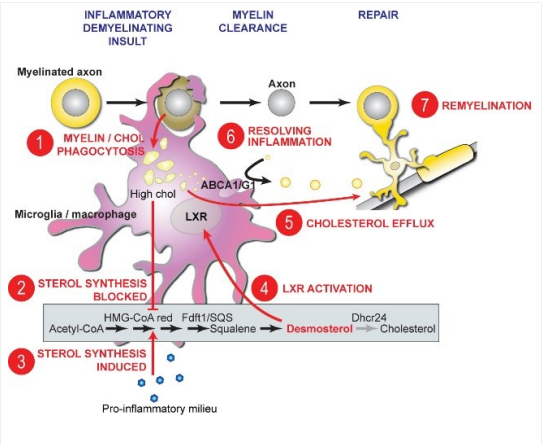

Working model of how microglia support repair of demyelinated lesions

T12-010A

The intricacies of remyelination failure problem: a novel alginate-based hydrogel to understand the impact of mechanical properties changes on oligodendrocyte differentiation

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With over 2.5 million people affected worldwide, multiple sclerosis (MS) represents a serious health, economic and social burden with no long-term suitable treatment. Remyelination failure in MS is a longstanding problem that remains to be solved. The formation of a glial scar containing reactive astrocytes overexpressing altered extracellular matrix (ECM) proteins contributes to a complete alteration of the microenvironment surrounding these cells. We hypothesize that oligodendrocytes (OLs) respond to these dynamic biomechanical changes altering their differentiation capacity, and in turn the remyelination is compromised. By tuning glial scar-mechanosensing mediated pathways one expects to be able to promote remyelination.

Here we propose a novel fully three-dimensional tissue-engineered model to study the impact of mechanical properties changes on OL differentiation. Alginate (ALG) was chosen as the polymer to mimic the extracellular milieu due to its biocompatible, non-toxic and tunable matrix mechanical properties.

ALG hydrogels were produced by combining modified ALG formulations containing the cell adhesive peptide RGD (GGGGRGDSP) or the matrix metalloproteinase sensitive peptide PVGLIG (GGYGPVG↓LIGGK). Primary rat oligodendrocyte precursor cells (OPCs) growing and differentiation was optimized by varying the alginate content (0.5-1.5%), PVGLIG and RGD concentration (40 – 400 μM) and polymer chain oxidation status.

The impact of the mechanical properties changes on OL differentiation was assessed by culturing OPCs within

increased alginate content hydrogels (1%, 2% and 3% wt/v). OL metabolic activity and differentiation (assessed by the expression of the myelin basic protein, MBP) was favoured in softer matrices (shear modulus, G* ~100 Pa) in comparison with low expression of MBP and decreased cell volumes for G* values around 350 Pa and 1300 Pa. Additionally, impaired OL differentiation was verified for hydrogels with similar stiffness values but with increased stress-relaxation times, which indicates an enhanced cellular behaviour in matrices with augmented capability of dissipating cell-induced forces.

To recreate a glial scar *in vitro*, primary rat astrocytes were also embedded within ALG matrices and found to extend long processes, with increased cellular complexity for high PVGLIG matrices. Activation with LPS/IFN-γ lead to an increase of Lcn2 and Col4 expression, strongly indicating that astrocytes acquire an astrogliosis-like phenotype.

By combining the culture of OLs and activated astrocytes within the ALG hydrogels we are expecting to understand the crosstalk between these cells and investigate the effects of matrix alterations provoked by astrogliosis processes on OL differentiation.

Acknowledgement

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T12-011A

A role of Schmidt-Lanterman Incisures for sustaining Schwann cell function in Charcot-Marie-Tooth Disease type 1A

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Schmidt-Lanterman Incisures (SLIs) are funnel-shaped cytoplasmic channels in the compact myelin internode of the peripheral nervous system. SLIs are thought to provide metabolic “shortcut routes” from the Schwann cell to the axon whereas experimental evidence for their function is largely missing. Schwann cell specific knock out of *vcl*, which encodes for the actin-binding protein Vinculin, results in a reduced number of SLIs without effecting radial myelination, motor behavior or electrophysiological measurements. In healthy Schwann cells, a reduced number of SLIs does not alter the phenotype and Vinculin conditional knockout mice can provide a useful model to study SLI function in health and disease of the peripheral nervous system. Peripheral neuropathies such as Charcot-Marie-Tooth disease type 1A (CMT1A) display an increase in SLI number. Deleting Vinculin specifically in CMT1A Schwann cells results in decreased SLI numbers and a deteriorated phenotype. Thus, in peripheral neuropathies an increase in SLIs appears beneficial, possibly due to more "shortcut routes" for metabolic support from the Schwann cell to the axon. Increased SLI numbers in CMT1A may compensate for metabolic needs of the diseased Schwann cell-axon unit while a reduction of SLIs in CMT1A leads to a lack of compensation and an overall deteriorated phenotype.

T12-012A

Parvalbumin interneuron myelination determines fast inhibition and cortical network rhythmicity

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Parvalbumin-positive (PV^+) GABAergic interneurons are a class of fast-spiking interneurons exerting powerful control of cortical microcircuit oscillations via rapid and temporally synchronized inhibition. Recent studies show that axons of PV^+ interneurons are myelinated, but the function sub-served by their myelination remains poorly understood. Here, we hypothesized that the loss of compact myelination impairs inhibition and cortical rhythm generation in the neocortex and used the electrophysiological recordings of PV^+ basket cell-layer 5 pyramidal neurons pairs combined with optogenetic activation of PV^+ interneurons in cuprizone-induced demyelination and a myelin mutant *shiverer* mice. Cuprizone treatment led to a ~2-fold reduction in the connection probability ($P < 0.0182$), consistent with a reduced number of perisomatic PV^+ and synaptotagmin-2^+ boutons in both cuprizone-treated mice ($P = 0.003$, $n = 7$) as well as in *shiverer* mice ($P = 0.0013$, $n = 17$). Paired recordings from PV-to-L5 pyramidal neuron pairs showed that the unitary inhibitory postsynaptic currents exhibited an increased failure rate and ~2-fold reduction in peak amplitude, consistent with a decline in the peak amplitude of the population inhibitory current evoked by optogenetically activated ChR2 expressed in cuprizone-treated PV-Cre mice ($P = 0.036$, $n = 8$). To test whether loss of fast inhibition changes cortical rhythms we chronically recorded *in vivo* the electrocorticogram and layer 5 local field potential in the primary somatosensory and visual cortex in freely moving awake mice. The results indicated a significant amplification in the theta band ($P < 0.0001$, n = 6) along with the appearance of large amplitude synchronized interictal spike discharges during quite wakefulness but not during locomotion behaviour. Furthermore, in awake cuprizone-treated mice, optogenetically entrained cortical gamma oscillations in were significantly abolished ($P = 0.0006$, $n = 8$). Finally, in order to test whether reduced PV-mediated inhibition underlies the hyper-synchronous oscillations and interictal spike discharges we applied long duration pulses of blue light activating PV^+ interneurons (1 sec pulse for 5 mins) or injected the allosteric $GABA_A$ modulator diazepam. In both conditions, theta power was reduced to control levels and interictal spike rate significantly reduced (optical; $P = 0.016$, $n = 6$, pharmacological; $P = 0.024$, $n = 6$). Taken together, our data suggest that compact myelination of PV^+ axons is required for the assembly and maintenance of fast perisomatic inhibition of pyramidal neurons, and its loss drives aberrant theta oscillations and neuropathological synchronized epileptic-like discharges in the neocortex.

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T12-013B

DNA methylation regulates the expression of the negative transcriptional regulators ID2 and ID4 during OPC differentiation.

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The differentiation of oligodendrocyte precursor cells (OPCs) into myelinating oligodendrocytes is a well-established process, coordinated by an intricate network of transcriptional regulators. Epigenetic mechanisms, such as DNA methylation, have been suggested to control this network. The exact mechanism by which DNA methylation influences the myelin regulatory pathway during OPC differentiation remains poorly elucidated though. Here, we identified both the DNA-binding protein inhibitors, *Id2* and *Id4,* as the main targets of DNA methylation during the differentiation of murine OPCs into mature oligodendrocytes. We show that these inhibitory transcriptional regulators display hypermethylation, concomitant with decreased expression levels, as OPCs differentiate into oligodendrocytes *in vitro*. Furthermore, by making use of epigenetic editing by CRISPR/dCas9-DNMT3a, we confirm that targeted methylation of *Id2/Id4* boosts OPC differentiation and *Mbp, Plp* and *Mobp* gene expression *in vitro*. Finally, we show that in a pathological context such as multiple sclerosis (MS), methylation as well as gene expression levels of both *ID2* and *ID4* are altered compared to non-neurologic control human brain samples. Taken together, our data reveal that DNA methylation regulates *Id2/Id4* within the transcriptional network that drives myelination during oligodendrocyte development, which does not only reveal new insights into oligodendrocyte biology, but could also lead to a better understanding of CNS myelin disorders, such as MS.

T12-014B

Enhancing myelin renewal reverses cognitive dysfunction in a murine model of Alzheimer's disease

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Severe cognitive decline is a hallmark of Alzheimer’s disease (AD), an age-related neurodegenerative disease for which treatment has remained elusive. In addition to grey matter loss, significant alterations in white matter pathology have been identified in patients with AD. To investigate the potential contribution of myelin dysregulation

to AD-related deficits in neuronal physiology and cognition, we characterized the dynamics of myelin generation and loss in the APP/PS1 mouse model of AD. Unexpectedly, we observed a dramatic increase in the rate of new myelin formation in APP/PS1 mice, reminiscent of the robust oligodendroglial response to demyelination. The increase in myelin generation was accompanied by significant degeneration of pre-existing myelin, resulting in overall decreased levels of myelination in the cortex and hippocampus of APP/PS1 mice as well as in postmortem tissue of individuals with AD. Genetically or pharmacologically enhancing myelin renewal, by oligodendoglial deletion of the muscarinic M1 receptor or systemic administration of the pro-myelinating drug clemastine, improved the performance of APP/PS1 mice in memory-related tasks. Furthermore, hippocampal sharp wave ripples – a neuronal correlate of memory consolidation – were increased in APP/PS1 mice with genetically enhanced myelin renewal. Taken together, these results identify myelin loss as a prominent feature of AD and demonstrate the potential of enhancing myelination as a therapeutic strategy to improve AD-related cognitive impairment.

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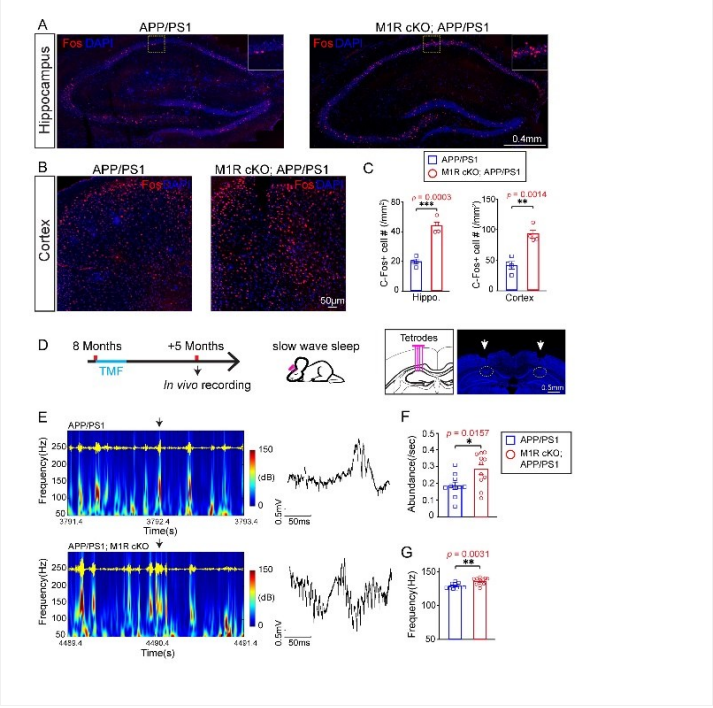

Figure 2. M1R deletion changes hippocampal SPW-Rs and firing activity of hippocampal pyramidal cells
A-C, Representative images and quantification of activated neurons by using Fos immunostaining (red) in the hippocampus **(A, C)** and cortex **(B, C)** of APP/PS1 and M1R cKO; APP/PS1 mice.**D,** Schematic diagram displaying the time course for tamoxifen induction and *in vivo* recordings. Representative images showing the location of tetrode implantation. Scale bar, 0.5mm; **E,** Hippocampal local field potential (LFP) spectra from the APP/PS1 and M1R cKO; APP/PS1 mice. **F-G,** Quantification of SPW-R abundance and frequency in the APP/PS1 mice and M1R cKO; APP/PS1 mice.

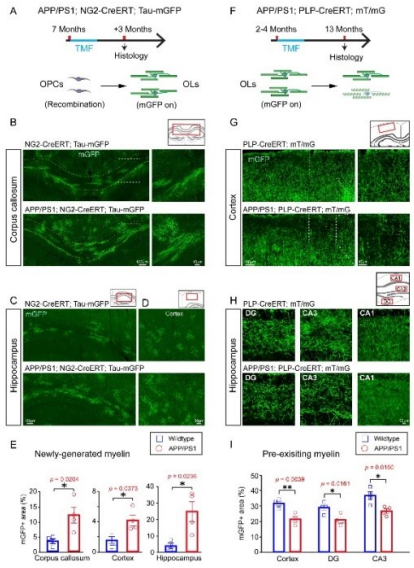

Figure 1. New myelin formation and degeneration are both elevated in APP/PS1 mice.
A, Schematic illustration showing the time course for tamoxifen induction and histology in the APP/PS1;NG2-CreERT;Tau-mGFP mice; **B-E,** Representative confocal images and quantification of mGFP positive myelin in the corpus callosum **(B, E)**, hippocampus **(C, E)** and cortex **(D, E)** of APP/PS1 mice and littermate controls. **F,** Schematic illustration displaying the time course for tamoxifen induction and histology in the APP/PS1;PLP-CreERT;mT/mG mice;**G-I,** Representative confocal images and quantification of mGFP positive myelin areas (green) in the cortex and hippocampus.

T12-015B

MicroRNA-125a-3p up-regulation impairs endogenous remyelination altering several pro-myelinating signaling pathways.

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Multiple sclerosis (MS) is a chronic immune-mediated demyelinating disease in which immune system attacks myelin, a fatty substance produced by oligodendrocytes, leading to abnormal transmission of nerve impulses. To become myelin-producing cells, oligodendrocyte precursors (OPCs) follow a very precise maturation process, finely regulated by transcription and epigenetic factors, and any alteration in this program can potentially contribute to dysregulated myelination, impaired remyelination and neurodegenerative conditions. In this respect, we recently identified miR-125a-3p as a new regulator of OPC maturation, showing that its over-expression impairs, whereas its inhibition stimulates this process.

Here, we show that miR-125a-3p is up-regulated in active lesions of MS patients, as well as in OPCs isolated from the spinal cord of chronic experimental autoimmune encephalomyelitis mice, but not in those isolated from the spontaneously remyelinating corpus callosum of lysolecithin-treated mice. To evaluate whether a sustained expression of miR-125a-3p in OPCs may contribute to defective remyelination, we modulated its expression in vivo after lysolecithin-induced demyelination, showing that lentiviral over-expression of miR-125a-3p impairs OPC maturation, whereas its downregulation accelerates myelin repair.

To shed light on the mechanism underlying this effect, we performed a microarray analysis on OPCs after miR-125a-3p over-expression and by using a combination of bioinformatic and pathway-focused approaches, we identified new miR-125a-3p targets, whose silencing contributes to impair OPC maturation, and several altered pathways, such as Wnt-signaling and those related to ECM and adhesion molecules, already known to be involved in OPC maturation and myelination.

Altogether, these results suggest that miR-125a-3p over-expression may negatively affect the remyelination process by altering several players and signaling pathways necessary to oligodendroglial cells to reach terminal maturation and become myelin-producing cells; thus we postulate that an antago-miRNA specific for miR-125a-3p may help to promote remyelination in demyelinating diseases.

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T12-016B

Timely Schwann cell division during migration drives peripheral myelination *in vivo*

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Schwann cells (SC) migrate along peripheral axons and divide intensively to generate the right number of cells prior to axonal ensheathment; however, little is known regarding the temporal and molecular control of their division, particularly during migration, and its impact on myelination. We report that Sil, a spindle pole protein associated with microcephaly is required for temporal mitotic exit of SC. In *sil*-deficient *cassiopeia* (*csp*$^{-/-}$) mutants, SC fail to radially sort and myelinate peripheral axons. Elevation of cAMP in *csp*$^{-/-}$ restores myelin ensheathment. Most importantly, we show a significant decrease in Laminin expression within *csp*$^{-/-}$ posterior lateral line nerve and that forcing Laminin2 expression in *csp*$^{-/-}$ fully restores SC ability to myelinate. We also discovered that SC have a restricted time window during which they have to divide, while migrating, in order to trigger myelination (Fig.). Thus, we unravel a novel and essential role for timely SC division during migration in mediating Laminin expression to orchestrate radial sorting and peripheral myelination *in vivo.*

Acknowledgement
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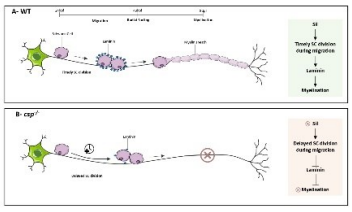

Summary scheme of the presented results
Summary scheme of the presented results. The spindle pole protein Sil tightly controls the temporal mitotic exit of Schwann cells. A, in Wild Type, a functional Sil allows the timely division of Schwann cells during migration, mediating laminin expression, thus myelination. B, in the absence of Sil (csp mutant), Schwann cell division is delayed, leading to important defects in laminin expression and a complete lack of myelin.

T12-017B

The IRE1/XBP1 pathway of the UPR modulates disease severity in Charcot-Marie-Tooth type 1B mice

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Myelin protein zero (Mpz or P0) is the most abundant protein in the myelin of peripheral nerves. In humans, P0S63del and P0R98C mutations cause mild and severe Charcot-Marie-Tooth (CMT) type 1B, respectively. Similar demyelinating neuropathies occur in transgenic mice carrying these mutations. Both P0S63del nad P0R98C mutant proteins are retained in the endoplasmic reticulum (ER) where they cause the activation of an unfolded protein response (UPR). The UPR is characterized by the activation of the PERK, ATF6 and IRE1 pathways. We have previously reported that the genetic and pharmacological targeting of the PERK pathway modulates disease severity in P0S63del mice, but the role of the other UPR branches remains to be investigated. To unravel the role of the IRE1 pathway in CMT1B, we generated new models of CMT1B mice in which the XBP1 gene, a key transcription factor downstream of IRE1, is deleted or overexpressed in Schwann cells specifically. We observed that the absence of XBP1 dramatically worsens hypomyelination as well as electrophysiological and locomotor parameters in young and adult P0S63del neuropathic animals. This suggests that the activation of XBP1 targets plays a critical role in limiting P0S63del toxicity, which cannot be compensated by other stress responses. Similarly, ablation of XBP1 in P0R98C mice further worsens the dysmyelinating phenotype and neurophysiological parameters, proving our findings in another CMT1B model. Remarkably, in both P0S63del and R98C mice overexpressing XBP1, we observed an improvement of some disease parameters, such as myelin thickness and nerve conduction velocities. Overall, these data demonstrate that the IRE1/XBP1 pathway has an essential adaptive role in P0S63del and P0R98C neuropathies and suggest that activation of this pathway can attenuate the severity of CMT1B disease and could prove beneficial for other neuropathies characterized by UPR activation.

T12-018B

Untargeted lipidomic analysis of brain myelin composition and changes during development

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Context of the study

Myelin, which is essential for the efficient conduction of action potentials along axons, owes its insulating properties to its high lipid content. Indeed, lipids represent approximately 70% of myelin dry weight, and any change in myelin lipid composition is susceptible to contribute to neurological disabilities. Thus, lipids could be used as sensitive markers of myelin status in physiological and pathological contexts.

Objectives and Methods

In this context, we have performed an exhaustive lipidomic analysis of mouse myelin using liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). Brain myelin was purified by magnetic activated cell sorting (MACS technology, Miltenyi) at two developmental stages: post-natal (P) days 15 and 40, representing respectively early and late myelination stages. Myelin samples were also compared to whole brain homogenates. In addition, O4+ oligodendrocytes were isolated at several post-natal stages to assess potential correlation between lipid homeostasis perturbation and change in gene expressions within specific lipid metabolic pathways.

Results

Comparison of purified myelin to whole brain highlighted an enrichment in characteristic myelin lipids. As expected, myelin was more abundant at P40 than at P15, which was reflected by a higher similarity in the composition of whole brain and purified myelin at this stage than at P15. Moreover, comparison of purified myelin between P15 and P40 allowed a careful description of changes in lipid composition during myelination, including the increase of several characteristic lipid sub-classes such as sulfatides and ethanolamine plasmalogens. Supporting these variations was the correlation with changes in the expression of several genes belonging to related lipid metabolic pathways, as evidenced by RT-qPCR in O4+ oligodendrocytes.

Conclusion and perspectives

We have optimized an approach to purify myelin and finely describe its lipid composition in a reproducible manner. In addition to improving our knowledge of myelination and myelin composition, this study may be regarded as a prerequisite for studying myelin alterations through its lipid content in various pathological contexts.

T12-019B

Imbalance of Neuregulin-Erbb2 pathway leading to altered myelin homeostasis in models of Charcot-Marie-Tooth diseaseType 4H

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Charcot Marie Tooth disease (CMT) is one of the most common inherited neurological disorders, affecting either axons from motor and/or sensory neurons or Schwann cells (SC) of the peripheral nervous system, or both. We previously identified mutations in *FGD4*, as responsible for CMT4H, an autosomal recessive demyelinating form of CMT, showing particular myelin abnormalities, called myelin outfoldings. *FGD4* encodes for FRABIN (FGD1-related F-actin binding protein), a GDP/GTP nucleotide Exchange Factor (GEF). In addition to its GEF activity, FRABIN can bind F-actin as well as phosphoinositides, through dedicated domains. This suggests that FRABIN could be involved in intracellular trafficking, and therefore might be essential to ensure proper supply of key signaling elements in PNS myelination process. In order to better understand the pathophysiology of CMT4H, in particular the role of FRABIN in the PNS myelination , we have developed a knock-out mouse model, with conditional ablation of *fgd4* in SC (cKO). In vitro, dorsal root ganglion (DRG)-SC cocultures from cKO embryos display myelin abnormalities similar to the myelin outfoldings observed in the sural nerves of CMT4H patients. Remarkably, these defects are also observed in sciatic nerves of cKO mice as soon as 3 months of age and increase over time. In

parallel we noticed an upregulation of components of the Neuregulin1 (NRG1)-type III- ErBB2/3 pathway, known as the main pathway inducing peripheral nervous system myelination. In parallel, using yeast two-hybrid experiments, we identified SNX3, a protein involved in endosomal trafficking, as a partner of FRABIN. Finally, we observed that preventing NRG1-ERBB2/3 binding, pharmacologically, using niacin abolishes myelin abnormalities and restores physiological myelination.

Our results demonstrate, for the first time, a dysregulation of NRG1-ErBB2/3 pathway in CMT4H pathology and highlight the pharmacological targeting of this pathway as a promising therapeutic approach for this demyelinating disease of the peripheral nervous system.

T12-020B

p38MAPKγ inhibits OPC differentiation during development and remyelination and is present in MS lesions.

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Multiple Sclerosis (MS) is usually characterized by primary demyelination followed by remyelination during early stages, failing in secondary and chronic progressive stages and with aging, as the environment becomes progressively more hostile. The reasons are likely to include factors that are both intrinsic and extrinsic to oligodendrocyte precursor cells (OPCs). Failure in OPC differentiation is an accepted cause of remyelination impairment which can be explained in part by inhibitory signals produced by the inflammatory environment. Thus, successful regeneration of myelin may occur only if we can favor remyelination in an inflammatory context.

We identified the minor stress-activated kinase, p38MAPKγ (p38γ) as a novel inhibitor of OPC differentiation during myelination and remyelination. *In situ* hybridization (ISH) experiments show that p38γ is ubiquitously expressed in the brain and downregulated during myelination. p38γ is expressed in PDGFrα$^+$ OPCs, NeuN$^+$ neurons, Iba1$^+$ microglia and GFAP$^+$ astrocytes but not in MBP$^+$ cells,. Mice deficient for p38γ exhibit premature appearance of oligodendrocytes (OLs) and myelin, which has normal morphology and thickness. Furthermore, the conditional deletion of p38γ in OPCs *in vivo* also produces premature appearance of OLs and myelin, indicating that precocious myelination in constitutive-null mice is mediated by a cell-autonomous effect in OPCs. *In vitro* experiments show that p38γ null OPCs mature faster than WTs and overexpression of p38γ in WT OPCs delay maturation.

During demyelination/remyelination by using the cuprizone (CPZ) model, both p38γ null and p38γ floxed-NG2CreER^{T2} mice have increased remyelination following 2 weeks of CPZ withdrawal.

Notably, microarray data shows that p38γ mRNA is enriched in white matter lesions from post-mortem MS patients. Thus, we investigated the expression of p38γ by ISH in leukocortical MS lesions and found that p38γ mRNA is enriched in demyelinated MS lesions. In addition, OPCs within the lesions express high levels of p38γ suggesting an inhibitory role of p38γ on OPC differentiation in MS

Because p38γ is induced in response to a variety of signals during inflammation and oxidative stress, is accumulated in MS lesions and delays remyelination in a mouse model of demyelination/remyelination, we propose that p38γ inhibition could be exploited as a therapeutic approach to promote remyelination in demyelinating diseases.

T12-021B

Myelin-dependent distribution of mitochondria in parvalbumin interneuron axons

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Parvalbumin-expressing interneurons (PV$^+$ INs) are critical for cortical rhythms and cognitive functions. Interestingly, recent work indicates that their proximal axons are myelinated. The myelin sheath facilitates saltatory propagation of action potentials and also transfers metabolites to axonal mitochondria, enabling internodal energy production. While it is well established that myelin loss in multiple sclerosis or experimental demyelination impacts mitochondrial integrity it remains unclear whether such mitochondrial changes occur in PV$^+$ interneuron axons. Here, we developed a Cre-dependent AAV methodology to fluorescently label mitochondria with GFP in genetically defined cell types. Stereotactic injections in the mouse somatosensory cortex were used to obtain single-cell labeling and detailed anatomical reconstructions to determine the subcellular distribution of mitochondria throughout the branches of either cortical layer (L)5 pyramidal neurons (*Rbp4-Cre* mice) or PV$^+$ INs (*PV-Cre* mice). We found that in both cell types, axonal mitochondria are significantly smaller and rounder than those found in dendrites, which was confirmed using publicly available electron microscopy data. To test the role of myelin on axonal mitochondria density and size we induced demyelination by feeding mice for 6 weeks a diet supplemented with the oligodendrocyte-selective toxin cuprizone. Detailed reconstructions of segments beyond the AIS and up to the 8th branch order of PV$^+$ axons showed that mitochondrial density in demyelinated proximal axonal branches was significantly reduced by ~20% (0.25 vs. 0.19 mitochondria/µm, n = 8 axons each, P = 0.01). Moreover, mitochondrial size was enlarged in demyelinated axons (0.43 vs 0.69 µm^2, n = 805 vs 1218 mitochondria from 5 cells each, $P < 0.05$). The total axonal length was unchanged and no structural abnormalities were apparent in demyelinated PV$^+$ axons. On the electrophysiological level, the action potential waveform of demyelinated PV$^+$ INs was unaffected, but demyelination reduced the action potential firing frequency (maximally 172 vs. 132 Hz, $P < 0.05$). In conclusion, these data show an AAV strategy that enables the cell-type specific analysis of mitochondria. The findings indicate that myelinated axonal regions in PV$^+$ interneurons are likely to actively recruit mitochondria.

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T12-023C

Learning induces periaxonal and nodal plasticity in the adult mouse brain

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Increasing neuronal activity, either by direct stimulation or through learning, promotes oligodendrogenesis and new myelin addition within the activated circuit. However, little is known about how mature oligodendrocytes respond to changes in neuronal activity. To investigate this, we trained adult mice (P60) to perform a spatial learning task (radial arm maze) over a 14-day period. We found that spatial learning increased node of Ranvier length by ~16% in the hippocampal fimbria. This change in node length was independent of oligodendrogenesis and appeared to be mediated by the subtle retraction of internodes elaborated by pre-existing mature oligodendrocytes. Additionally, the average g-ratio of axons within the fimbria increased by ~9%, primarily due to a ~29% decrease in the space between the myelin the axon known as the periaxonal space. Computational modelling and electrophysiological analyses revealed that these adaptive periaxonal and nodal changes worked synergistically to increase action potential conduction velocity along myelinated axons in the fimbria-fornix pathway by ~43%. Remarkably, myelinated axon conduction velocity correlated with radial arm maze performance i.e. mice with faster conduction velocities made fewer errors in the radial arm maze task. These data suggest that subtle changes to axon-myelin structure and action potential conduction velocity may facilitate learning.

T12-024C

Glial precursor cells generated from rat mesenchymal cells respond to repurposed drugs, enhancing their differentiation, metabolism and ensheath abilities.

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Central Nervous System (CNS) demyelinating diseases (for instance, multiple sclerosis) are characterized by failures in migration, proliferation and/or differentiation of oligodendrocyte precursor cells (OPCs), which produce lack of myelination. We have recently generated functional induced OPCs through direct lineage conversion by overexpressing Sox10 + Olig2 + Zfp536 (SOZ) in adult rat adipose tissue-derived stromal cells (ADSC). These iCPOs express several oligodendroglial markers, such as O4, O1, MYRF, QKI-7, PLP1, MAG, MOG, MBP, and they have the ability to proliferate, migrate and differentiate into mature oligodendrocytes.
Nevertheless, recent evidences in our group have demonstrated that, under particular culture conditions, this procedure is also efficient to generate astrocytes, Schwann cells and olfactory ensheathing like-cells as shown by immunofluorescence and/or electron microscopy. Quantitative analysis of several lineage markers (GFAP, myelin P0, periaxin, and p75NTR) by RT-qPCR have molecularly confirmed these phenotypes.

Furthermore, we show that several repurposed drugs such as kainate, benztropine, miconazole, taurine, clobetasol or baclofen promote iOPCs differentiation, bioenergetics and ensheathing abilities as they directly influence iOPCs to favour myelination. To demonstrate these effects we performed morphological complexity fractal analysis, metabolic flow analysis (Seahorse), mitochondrial dynamics (Mitotracker), RT-qPCR and confocal/electron microscopy. Benztropine and baclofen postulate as hit compounds, since they enhanced to a greater extent the analyzed parameters, similarly as it is observed when using brain-derivedOPCs. Taurine also potentiates the effects of benztropine on morphological complexity, bioenergetics and both mitochondrial membrane potentials and numbers.

This study supports the potential use of these induced glial precursor cells (iGPCs) to test *in vitro* the molecular and cytological effects of pro-myelinating and repurposed drugs. These iGPCs may also constitute a reliable source of cells for studying other CNS pathologies caused by the impairment of astrocytes or Schwann cells, such as in Alexander disease and peripheral neuropathies.

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T12-025C

Unraveling the role of SUMOylation peripheral myelination

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The mechanisms that govern myelination in the peripheral nervous system are not completely understood. Post-translational modifications of proteins are necessary for peripheral myelination, and malfunction of these pathways leads to neuropathies. Whether SUMOylation, covalent attachment of Small Ubiquitin-like Modifier (SUMO) proteins to the substrate, is involved in the formation of myelin and/or in the pathophysiology of peripheral neuropathies is not known.

We previously identified SUMO2 as a novel protein that may be involved in the early interaction between axons and Schwann cells, along with 10 immediate neighbors in the interactome. Using a prediction algorithm, we found that ~70% of the proteins enriched in Schwann cell pseudopods extending towards neuronal membranes may be SUMOylable in consensus and non-consensus SUMOylation sites, whereas ~60% of them may interact with SUMO proteins through SUMO interaction motifs (SIMs).

Here we show that gene targeting and pharmacological manipulation of SUMOylation affect Schwann cell myelination *in vitro*. SUMO2 is also involved in Schwann cell elongation upon early interaction with axons and is necessary for Schwann cell survival. Free and conjugated SUMO 1, 2 and 3 proteins as well as the conjugating enzyme UBC9 are highly expressed in sciatic nerves during development. Interestingly, some of the master regulators of Schwann cell development and peripheral myelination have been previously found to be SUMOylated

in other contexts and cell types. We observed that the Rho GTPase Rac1, which is necessary for radial sorting and myelin formation, interacts with SUMO2 in developing mouse sciatic nerves. Other SUMO substrates are currently under investigation. Finally, abnormal SUMOylation was observed in peripheral neuropathies, such as Charcot-Marie-Tooth type 1B. Transgenic mouse models will help us understand the role of SUMOylation and its targets in Schwann cell function and peripheral myelination in health and disease.

T12-026C

Targeted nanomedicines to stimulate the differentiation of oligodendrocyte progenitor cells in the scope of multiple sclerosis

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Impairment of oligodendrocytes progenitor cell (OPC) differentiation into oligodendrocytes and chronic inflammation are one the key determinants of poor remyelination observed in diseases such as multiple sclerosis (MS). Although numerous molecules are known to stimulate OPC differentiation and to reduce chronic inflammation, their potential as therapeutic drugs for MS is often hindered by limited access to the targeted cells. In addition, some of these molecules are lipophilic, which limits their solubility in physiological fluids and thus impairs their bioavailability. For instance, retinoic acid (RA) and calcitriol (Cal), two lipophilic drugs, can reduce chronic inflammation and stimulate remyelination by inducing OPC differentiation[1, 2]. A promising approach to improve the delivery of these lipophilic molecules to the CNS is to combine drugs with colloidal carriers, such as nanoparticles. Indeed, nanoparticles, in particular lipid nanocapsules (LNC), significantly improve solubility of lipophilic drugs. Moreover, the encapsulation into LNC protects molecules from degradation and can be designed to optimize drug loading and transport across epithelial barriers. We herein propose to encapsulate RA and Cal in LNC and to target them toward OPCs. As the platelet-derived growth factor receptor α (PDGFRα) is mostly expressed in the CNS on the surface of OPCs, we chose to achieve targeting by grafting an anti-PDGFRα antibody on the surface of the LNC. We hypothesized that targeting would allow for better accumulation of drug-loaded LNCs in the OPCs, stimulating their differentiation and limiting off-target effects. We successfully encapsulated RA and Cal inside LNCs and evaluated the stability of the formulations over time (size by Nanosizer and RA and Cal content by HPLC-UV). We found that RA-LNC and Cal-LNC were stable up to 8 weeks at 4°C in the dark. Next, we evaluated the impact of RA and Cal-loaded LNC on pro-inflammatory cytokine secretion. Drug-loaded LNC had a significant impact on IL-6, TNFα and MCP1 production. Next, we assessed the effect of these same nanoparticles on an OPC cell line (CG-4) and measured differentiation by measuring myelin basic protein (MBP) expression (RT-qPCR). RA- and Cal-loaded LNC (at 200 nM of RA and 50 nM of Cal) and their combination (RA+ Cal) significantly increased MPB gene expression, where Cal-LNC+ RA-LNC > Cal-LNC > RA-LNC > LNC. We successfully grafted anti-PDGFRα on the surface of LNCs and observed their interaction with soluble PDGFRα (ELISA). Our next experiment will be to evaluate if the anti-PDGFRα grafting at the LNC surface can enhance their association with rat primary OPCs (FACS).

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T12-027C

Clemastine impairs myelin development

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Abnormalities in myelination are associated to behavioral and cognitive dysfunction in neurodevelopmental psychiatric disorders. We detected a severe reduction of myelination in both white and gray matter in a contactin-associated protein-like 2 (Caspr2) deficient mouse model of autism. Thus, therapies to promote or accelerate myelination could potentially ameliorate symptoms in mental illness. Clemastine, a histamine H1 antagonist with anti-cholinergic properties against Chmr1, is the most promising drug with promyelinating properties today (Mei et al., 2014). Clemastine penetrates the blood-brain barrier efficiently and promotes remyelination not only in animal models of multiple sclerosis, but also in patients. Here, we studied the role of clemastine in oligodendrocyte lineage during development. Chronic treatment of mice with clemastine induced an increase in oligodendrocyte differentiation in corpus callosum and cerebral cortex. However, despite the increase in the number of oligodendrocytes, conduction velocity of myelinated fibers (N1) in the corpus callosum was significantly decreased in clemastine-treated mice. Confocal and electron microscopy studies showed a reduction in the number of myelinated axons and nodes of Ranvier as well as a reduction of myelin thickness in corpus callosum. To understand the mechanisms leading to the impairment of myelin formation in the presence of an excess of myelinating oligodendrocytes, we focused on Chmr1-expressing microglial cells. This subset of microglia displayed a more branched morphology and a reduced expression of the pro-inflammatory marker iNOS in clemastine-treated mice. Altogether, these data suggest that clemastine is not a useful drug to promote myelination during development. Further studies are needed to clarify the role of microglial cells on developmental myelination.

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T12-028C

A critical role for autophagy in oligodendrocyte maturation and myelin formation

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(Macro)autophagy comprises a conserved lysosome-dependent catabolic pathway, facilitating the degradation of cytoplasmic proteins and damaged organelles. Although a few studies implicate autophagy in CNS demyelinating pathologies, its role, particularly in oligodendrocytes and CNS myelin, remains poorly studied.
In our study, we aim to shed light on this role, using both *in vitro* and *in vivo* approaches. *In vitro*, pharmacological inhibition of autophagy, using the highly selective autophagy kinase ULK1 inhibitor SBI-0206965, resulted in a maturation delay of myelin-producing oligodendrocytes. Furthermore, primary cultures of oligodendrocytes where autophagy is genetically ablated revealed severe defects in myelin sheet formation and altered cellular distribution of major myelin protein constituents. We are currently examining the role of autophagy *in vivo*, utilizing a new conditional mutant mouse line, in which autophagy is specifically ablated in the CNS myelinating glial cells after tamoxifen administration (*plp-Cre^{ERT2}; atg5 $^{fl/fl}$*). Biochemical and electron microscopy analysis of this mouse line has revealed differences in myelin protein levels, as well as morphological alterations in cKO animals compared to age-matched controls.
Our findings suggest that autophagy is an indispensable mechanism for oligodendrocyte maturation and proper myelin formation.

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T12-029C

Proteome profile of myelin in the zebrafish brain

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The velocity of nerve conduction along vertebrate axons depends on their ensheathment with myelin. Myelin membranes comprise specialized proteins well characterized in mice. Much less is known about the protein

composition of myelin in non-mammalian species. Here, we assess the proteome of myelin biochemically purified from the brains of adult zebrafish (*Danio rerio*), considering its increasing popularity as model organism for myelin biology. Combining gel-based and gel-free proteomic approaches we identified >1000 proteins in purified zebrafish myelin, including all known constituents. By mass spectrometric quantification, the predominant Ig-CAM myelin protein zero (MPZ/P0), myelin basic protein (MBP) and the short chain dehydrogenase 36K constitute 12%, 8% and 6% of the total myelin protein, respectively. Comparison with previously established mRNA-abundance profiles shows that expression of many myelin-related transcripts coincides with the maturation of zebrafish oligodendrocytes. Zebrafish myelin comprises several proteins that are not present in mice, including 36K, CLDNK and ZWI. However, a surprisingly large number of ortholog proteins is present in myelin of both species, indicating partial evolutionary preservation of its constituents. Yet, the relative abundance of CNS myelin proteins can differ markedly as exemplified by the complement inhibitor CD59 that constitutes 5% of the total zebrafish myelin protein but is a low-abundant myelin component in mice. Using novel transgenic reporter constructs and cryo-immuno electron microscopy we confirm the incorporation of CD59 into myelin sheaths. These data provide the first proteome resource of zebrafish CNS myelin and demonstrate both similarities and heterogeneity of myelin composition between teleost fish and rodents.

T12-030C

Diversity of CNS myelin protein composition between humans and mice associated with heterogenous oligodendrocyte mRNA abundance profiles

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Myelin is commonly assessed in the brains of experimental mice as a model for humans; however, it remained unclear to what extent their protein composition is actually equal. Here we use label-free mass spectrometry for deep quantitative coverage of the proteome of myelin purified from the white matter of human subjects post-mortem. By comparison with previously established datasets, the protein composition of myelin in humans correlates to a considerable extent with that in c57Bl/6N mice, including the relative abundance of the major structural myelin proteins PLP, MBP, CNP, CLDN11, MAG and MOG. However, multiple other myelin proteins were identified exclusively or predominantly in either mouse or human myelin, indicating diversity of myelin composition among mammalian species. This is exemplified by tetraspanin-2 (TSPAN2), a known myelin constituent in mice and rats but not identified in human myelin. Assessing published scRNA-seq datasets, this heterogeneity of the myelin proteome correlates with divergent mRNA abundance profiles. Indeed, mouse and human oligodendrocytes display principally different expression of Tspan2 and other myelin-related transcripts. Our data point to evolutionary specialization of the molecular profiles of mammalian oligodendrocytes and myelin with implications for cross-species translation of knowledge regarding neuropathology in myelin-related disorders.

T12-031C

Bioimaging of Microglia and Myelin Clearance during demyelination Using the Fluorescent BASHY Platform

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Multiple Sclerosis (MS) is an inflammatory, demyelinating disease of the Central Nervous System characterized by the presence of demyelinated regions of myelin degradation with accumulated myelin-toxic lipid debris. For remyelination to be effective, such debris must be cleared by microglia. That is why the imaging of myelin clearance *in vivo* must be fundamental to evaluate lesion progression and assess MS disease course. Recently, using fluorescent Boronic acid-based complexes - BASHY dyes – we could accurately discriminate different lipid structures and specifically identify nonpolar lipid structures. Therefore, we aimed to address the ability of BASHY to label myelin-lipid fragments and their clearance in the context of demyelination.

We used *ex vivo* cerebellar organotypic slice cultures (COSC) from 10 postnatal rats treated with lysophosphatidylcholine (LPC, 0.5mg/mL), to induce demyelination. In parallel, microglia cultures were incubated with BASHY-stained myelin debris. Brain slices and microglial cells were immunostained to evaluate BASHY colocalization with myelin debris and also its specificity for phagocytosing cells. Additionally, mice were induced with Experimental Autoimmune Encephalomyelitis (EAE), and injected with BASHY probe [100ul(1mM)/20g body weight]. Both EAE and control animal brains were analyzed by immunohistochemistry and flow cytometry analysis to evaluate BASHY-positive microglia and BASHY's presence within demyelinated lesions.

Firstly, we observed that BASHY labeling significantly increases in LPC-treated COSC (2.88-fold, $P<0.05$), and stains cholesterol-rich myelin debris, corroborated by the co-localization with the cholesterol marker – filipin. Moreover, BASHY molecule was selectively internalised by Iba1-expressing microglia/macrophages, with an increased presence among the amoeboid/phagocytic/activated cells ($P<0.001$). Additionally, we proved BASHY's excellent stability upon microglia phagocytosis, accompanying lipid delivery into lysosomes and lipid droplets, observed by BASHY colocalization with Lysotracker® and Nile Red dye, respectively. Finally, *in vivo* studies identified the presence of BASHY fluorescence within demyelinating lesioned areas of EAE-mice, and the co-localization with lesion-associated Iba1-positive foamy cells, particularly evidenced at the peak of the disease.

Here, we describe a newly synthesized fluorescent molecule, BASHY, with high specificity for myelin debris and highly selective for myelin phagocytosing cells. This new tool will now be further optimized to be used non-invasively to identify resolving MS demyelinating lesions, as indicators of disease stage/progression, either in longitudinal pre-clinical studies using live animals, but also in MS patients clinical monitoring.

Acknowledgement

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T12-032C

The essential role of the thyroid hormone transporter MCT8 in oligodendrocyte maturation and myelination

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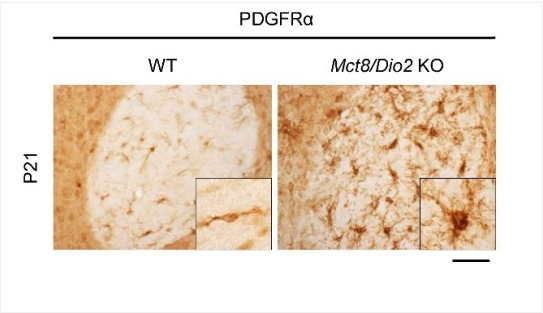
Oligodendrocytes are glial cells that play a crucial role in the CNS, where they produce the myelin sheaths that insulate axonal processes of neurons enabling saltatory conduction of action potentials and providing metabolic axonal support. Oligodendrocyte maturation and myelination are finely regulated processes that require key trophic signals important for growth and metabolism. Thyroid hormone (TH) is a potent signal that regulates oligodendrocyte maturation, myelination, and oligodendroglial synaptic interactions. TH transport across the blood-brain barrier and cellular membranes is mediated by a specific transmembrane transporter, the monocarboxylate transporter 8 (MCT8). Dysfunction of MCT8 results in impaired TH uptake in the developing brain that leads to inherited hypomyelination and psychomotor disabilities in the X-linked Allan-Herndon-Dudley syndrome (AHDS) or MCT8-deficiency. Even though the altered myelin status in AHDS patients is one of the main hallmarks of the disease, it is still a matter of debate whether there is a permanent hypomyelination or a delay on myelination that is restored later in life.

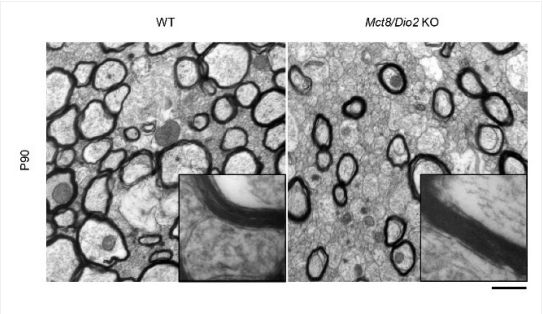
To address this point, we have made use of multiple approaches to study myelination processes in *Mct8/Dio2* knockout mice (KO), an already validated model for AHDS, from postnatal to adult stages, to gain new insight into the pathophysiological mechanisms of AHDS and the effects of TH on myelination.

Myelination was studied histologically by assessing the content of myelin proteins and lipids. These studies showed persistent myelination defects in the brain of *Mct8/Dio2* KO mice and were also in agreement with observations at the ultrastructural level showing severely decreased percentage of myelinated axons in the *Mct8/Dio2* KO mice brain using transmission electron microscopy analyses. Myelination was also assessed by Magnetic Resonance Imaging techniques, showing brain microstructural alterations in parameters used to evaluate myelinating disorders which, together with the histological data, corroborates that *Mct8/Dio2* KO mice replicate the myelination impairments reported in the patients and shows that these alterations are persistent. Data obtained on myelination led to the study on oligodendroglial dynamics, showing altered proliferation and differentiation patterns from oligodendrocyte precursor cell stages.

Myelination in *Mct8/Dio2* KO mice is altered from early developmental stages, when also oligodendroglial dynamics suffer several impairments, and these alterations persist throughout later stages with only partial recovery. Altogether, these data provide new understanding on the effects of TH on myelination, and on the pathophysiological mechanisms underlying MCT8 deficiency to design and evaluate possible future treatments.



Altered proliferation patterns from oligodendrocyte precursor cells in the Mct8/Dio2 KO mice brain.
Altered oligodendroglial development in *Mct8/Dio2* KO mice. Brain coronal sections of P21 WT (n = 4) and *Mct8/Dio2* KO (n = 4) mice were immunostained with anti-PDGFRα. Representative images depict the immunostaining pattern in the anterior commissure. PDGFRα immunopositive cell density is increased in the *Mct8/Dio2* KO mice. Insets depict PDGFRα positive cells in both WT and *Mct8/Dio2* KO mice. *Mct8/Dio2* KO mice OPCs show an aberrant increase in size and branching as compared to WT.



Severely decreased percentage of myelinated axons in the Mct8/Dio2 KO mice brain.
Altered myelination pattern in *Mct8/Dio2* KO mice. Ultrathin sections of the medial part of the cc were analyzed by electron microscopy at P90 (n = 6). The number of myelinated axons was severely decreased in *Mct8/Dio2* KO mice as compared to WT animals. No differences in the ultrastructure of the myelin sheaths were observed between both genotypes as seen in the higher-magnification insets.

T12-033C

Mechanisms controlling neuroblasts migration and reprogramming during myelin repair.

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Presence of neural stem cells (NSC) and progenitors is the basis of regenerative mechanisms in the adult brain. This potential of spontaneous repair is particularly promising for the treatment of neurodegenerative diseases such as multiple sclerosis. Indeed, following myelin damage in adult rodent brain, a self-repair process is observed via the formation of new oligodendrocytes (OLG). Two sources of cells participate to this repair process: OLG progenitors disseminated in the cerebral parenchyma and NSC located in the subventricular zone. Under physiological conditions, these NSC divide and mainly generate neuroblasts (NB) that migrate "in chain" (collective migration) along the rostral migratory stream to integrate the olfactory bulb and differentiate into interneurons. Under demyelinating conditions in mice, some NB leave their migration path and migrate toward the lesion as isolated cells **[1]**. This change in migratory behavior is associated with NB reprogramming, which transform into OLG and participate in myelin repair **[2, 3]**.

We showed, using genetic lineage tracing in different demyelination mouse models and transcriptomic analysis, that this fate change occurs through a direct cell reprogramming. It involves the tight control of several key processes including fate determination transcriptional programs and cell migration. We hypothesized that NB emigration and fate conversion is regulated by a mechanism related to the epithelial-mesenchymal transition (EMT). Many studies demonstrated that EMT is essential for the migration of stem/progenitor and cancer cells. We found that some EMT transcription factors are strongly induced in NB after lesion and interestingly, some are also involved in OLG formation. We are testing their functional implication in NB mobilization during myelin repair.

Since EMT is also controlled by tissue stiffness during developmental and regenerative process **[4]** and that OLG differentiation is impacted by mechanical forces **[5]**, we are also studying how matrix properties influence NB behavior. Our preliminary results using explants cultured in hydrogels indicate that a reduced matrix stiffness promotes change in NB migratory behavior, from collective (chain migration) to isolated cells, and cell reprogramming in vitro. In addition, recent work reported altered tissue stiffness in CC after demyelination **[6]**. We are testing how demyelination modify matrix stiffness in vivo using an atomic force microscope to understand how mechanical properties impact NB migration and fate conversion.

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T12-034D

Microglial interaction at nodes of Ranvier in health and repair

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Microglial cells are the resident immune cells of the central nervous system (CNS) and key players in brain homeostasis and plasticity. In Multiple Sclerosis (MS), an inflammatory disease of the CNS, activated microglia either promotes tissue damage or favors neuroprotection and myelin repair. The mechanisms of microglia communication with the different neuronal sub-cellular structures remain largely unknown. Here, we identify nodes of Ranvier as a direct and stable site of interaction between microglia and axons, in both mouse and human tissue. Using dynamic imaging, we highlight the preferential interaction of microglial processes with nodes of Ranvier, along myelinated fibers. We show that microglia-node interaction is modulated by neuronal activity and associated with K^+ release. Disrupting either axonal K+ flux or K+ homeostasis of microglia, following demyelination, polarizes microglia towards a pro-inflammatory phenotype, reduces microglial expression of pro-remyelinating factors and decreases remyelination rate. Taken together, these findings identify the node of Ranvier as a major site for microglia-neuron communication along myelinated and remyelinating fibers.

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T12-035D

The role of myelination in layer 5 corticothalamic feedback

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Myelination is essential for temporally precise and rapid action potential (AP) propagation. There is emerging evidence that both the myelin sheath properties and anatomical dimensions of nodes of Ranvier (noR) can be dynamically changed by experience and activity, causing different temporal delays across myelinated axons. In the somatosensory whisker system, the information transfer via projections from layer 5 (L5) pyramidal neurons to the posteromedial nucleus of the thalamus (PoM) plays a powerful role in providing feedback information to thalamic neurons, who can act as coincidence detectors of synchronized activity. Here, we studied specifically the role of L5-to-PoM myelination for precise timing of corticothalamic feedback *in vitro* and *in vivo.* Using a retrograde AAV targeting approach in combination with a L5-specific Cre-driver mouse line (*Rbp4-Cre*) and immunofluorescent stainings against nodal compartments (Caspr, Ankyrin G), we investigated morphological parameters of nodes and

internodes in different regions of the corticothalamic pathway, including the cortex, corpus callosum, striatum and thalamus. In control mice we observed regional differences in myelin patterns: while the internode length was significantly longer in the striatum in comparison to the cortex and thalamus ($P < 0.01$, $n > 70$), the nodes were ~ 0.5 µm longer in the cortex ($P < 0.05$, $n > 20$). After 6 weeks of cuprizone-induced demyelination, we observed a strikingly diverse pattern of disassembly of nodal compartments. NoR either lacked one or both paranodes. This observation was most prominent in the cortex (~20% of the noR), but also occurred in the striatum and thalamus. In the white matter, paranode lengths were significantly increased, indicating impaired node clustering. In contrast to axonal properties, the apparent intrinsic excitability and action potential firing of thalamic PoM neurons was not significantly affected by demyelination ($n > 13$). Next, we investigated whether the observed region-specific morphological diversity would affect information transmission. Using optogenetic stimulation of ChR2-labelled L5-to-PoM axons in corticothalamic acute brain slices in combination with whole-cell patch-clamp recordings of thalamic neurons, we identified a reduced conduction velocity in demyelinated L5-to-PoM axons (1 m/s vs 0.2 m/s, $P < 0.05$) and a significant and frequency-dependent impaired synaptic activation (at 20 Hz, 30% reduction, $P < 0.01$). In order to understand whether and how information transfer is affected *in vivo* we are employing juxtacellular recordings of L5 pyramidal neurons in combination with Neuropixels probes to record whisker-evoked activity in head-fixed mice.

Acknowledgement

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T12-036D

Improved Performance of Neuron-Free Microfibre Assay to Assess Myelin Sheath Formation

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Many different *in vitro* tools have enabled researchers to gain a much stronger understanding of oligodendrocyte biology.2D cultures of isolated primary oligodendrocyte precursor cells (OPCs) have shed light on the process of differentiation and using these cells in co-culture with neurons have helped shape our understanding of oligodendrocyte-neuron interactions. Recent work from several labs including ours has developed 3D oligodendrocyte culture systems using polymer fibres to mimic axons that facilitate the ability to observe myelin sheath formation. These fibre cultures represent a powerful tool to gain mechanistic insight into oligodendrocyte-specific regulation of myelin sheath formation. Being neuron-free, they have been used to investigate the intrinsic properties of oligodendrocytes and have the potential to be used for drug screening assays for compounds that enhance myelin formation specifically. They also allow for the direct quantification of the number and length of sheaths formed by single myelinating oligodendrocytes. However, they can be very sensitive to reagents and protocol changes, yielding inconsistency in myelin formation from culture-to-culture. We therefore set out to

optimize this assay through assessing the impact of both physical and molecular factors. Specifically, we highlight the importance of physical properties such as microfibre stiffness and the availability of iron in the myelination media for successful fibre cultures. We demonstrate that, once several physical and molecular factors are considered, fibre cultures yield consistent and reproducible results and represent a strong tool for assessing myelination *in vitro*.

T12-037D

Neocortical pyramidal cell myelination is predicted by local axonal morphology in mouse and human

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Myelin functions to enhance axonal conduction velocity and provide metabolic support. The myelination profile of pyramidal cells is highly diverse and may be related to their electrical activity and long-range connectivity. However, there is still insufficient knowledge about the cellular determinants underlying the distribution of internodes along neocortical pyramidal cell axons. Our recent studies in parvalbumin-positive interneurons have shown that local axonal morphology is highly predictive of segmental myelination. Whether pyramidal cell myelination adheres to similar parameters or other mechanisms are involved, is still unknown.

Here, we investigated the relationship between pyramidal cell axonal morphology and myelination in layer II/III of mouse somatosensory cortex, as well as layer II/III neocortical pyramidal neurons from human *ex vivo* neurosurgically resected tissue. We performed whole-cell patch clamp recordings together with biocytin labelling and structured illumination microscopy (SIM). We measured the axonal calibre and interbranch distance of myelinated and unmyelinated segments along the pyramidal cell axons. Using receiver-operating characteristic (ROC) analysis, we found that the joint combination of diameter and axonal segment length was highly predictive of segmental myelination, and a better predictor than either parameter alone. The optimally predictive thresholds for axonal diameter and interbranch distance in mice were 237 nm and 19.7 µm, respectively (AUC 0.98). Similar to the results in mice, the optimally predictive thresholds for human pyramidal cell axonal diameter and interbranch length were 269 nm and 19.0 µm, respectively (AUC 0.91).

Our data indicates that similar to neocortical fast-spiking interneurons, the bivariate metric of local axonal morphology involving both calibre and interbranch distance is an important determinant of segmental myelination along mouse and human neocortical pyramidal neurons.

T12-038D

Heme metabolism regulates oligodendrocyte differentiation and myelination

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Iron is highly enriched in mature oligodendrocytes in the central nervous system (CNS) and dysregulated CNS iron homeostasis is associated with many neurodegenerative diseases. It is hypothesised that iron is required to support high oligodendrocyte metabolic demands during differentiation and myelin maintenance. Using bioinformatic analysis of publicly available single cell RNA-seq datasets, genes associated with iron storage and iron transport showed robust expression in oligodendroglial lineage cells. In parallel we examined Heme metabolic pathways, showing heme synthesis and import associate with myelinating oligodendrocytes. Fluorescent in situ hybridization confirmed that the Heme transporter *Hrg1* (*Slc48a1*) is expressed in nearly all mature oligodendrocytes in the adult CNS. Deletion of *Slc48a1* resulted in significant increases in cortical and hippocampal myelination in adult CNS, explained by increased numbers of mature (*Ermn*$^{+}$) oligodendrocytes. Iron histological staining showed decreased iron content in the corpus callosom of *Slc48a1* deficient mice, demonstrating that heme catabolism contributes to oligodendrocyte iron. Pharmacological inhibition of heme degradation in vitro did not alter viabilities of OPCs or oligodendrocytes. Heme synthesis inhibitors showed specific toxicity to oligodendrocytes and not OPCs and thereby preventing differentiation of OPCs to mature oligodendrocytes. Addition of Hemin, to OPCs and oligodendrocytes showed oligodendrocytes are more resistant to Hemin toxicity than OPCs and the toxicity is dependent on oxygen concentrations. These results show the importance of Heme metabolism in regulating CNS numbers of oligodendrocytes and their levels of iron.

T12-039D

Use of peptide-fluorophore conjugates to asses putative targeting to glia of peptide-drug hybrid molecules as a new therapeutic approach for Multiple Sclerosis.

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Demyelinating diseases such as Multiple Sclerosis (MS) are a collection of pathologies that involve the degradation of the myelin sheath of the neurons, resulting in severe handicaps for neurological physiology. Oligodendrocyte precursor cells (OPCs) are the cells that upon differentiation and maturation (re)generate the myelin necessary for the correct function in central nervous system (CNS). Thus, finding ways to pharmacologically stimulate OPCs

maturation into myelinating oligodendrocytes (OLs) is considered one the of the great challenges in MS treatment. Previous work from our group has shown the effect of compounds that enhance this (re)myelinating process [1].
However, one of the additional key challenges on systemically administered drugs is finding strategies to deliver them into their specific target organs or cells. In this sense, small peptides have previously exhibited promising features as target-specific interactors [2], thus becoming potentially interesting carriers in hybrid molecule (carrier-drug) treatment strategies.
In this work, we utilized *ad hoc* designed small peptides, CTB2.20 and CTB 2.21 labelled withfluorophore Cy3 to validate the specificity of those peptides delivering small molecules into OPCs, both *in vivo* and in different cell cultures.

Acknowledgement
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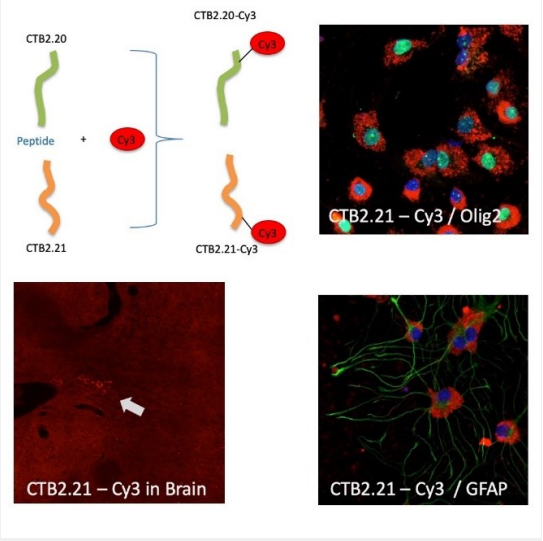

Graphical Abstract
Depiction of the concept behind the used hybrid molecule (peptide-Cy3,) and some preliminary results with *in vivo* brain (bottom-left, arrow points Cy3 signal at cerebellar peduncles region) and mixed culture from P7 mice stained either for Olig2 (OPCs, top-right) or GFAP (astroglia, bottom-right).

T12-040D

ApTOLL: a new aptamer to recover myelin in Multiple Sclerosis

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Multiple sclerosis (MS) is a degenerative, autoimmune and chronic disease of the central nervous system (CNS) that constitutes the second cause of neurological disability in young adults. It is characterized by the loss of oligodendrocytes and, therefore, myelin, both in the white and in the gray matter. On the other hand, the autoimmune component that underlies the pathology of MS is the promoter of the processes of inflammation, demyelination, and damage to the axonal network, where the Toll-like type 4 receptor (TLR4) and proinflammatory signaling that triggers its activation plays a crucial role. In this sense, the innovative ApTOLL molecule has been developed with aptamer technology and seeks to antagonize the TLR4 in order to achieve an immunomodulatory and anti-inflammatory effect. ApTOLL is a single chain DNA aptamer that supposes a novel strategy, both for its molecular nature and for its mechanism of action, for the treatment of diseases with an important inflammatory component such as MS.
In this research, the immunomodulatory and remyelinating effect of four doses of ApTOLL (0.45 mg/kg, 0.91 mg/kg, 1.82 mg/kg, and 3.6 mg/kg) has been determined for the study of the therapeutic dose of this compound in the Experimental autoimmune encephalomyelitis (EAE) model of MS. A clear reduction in the clinical score of animals treated with ApTOLL with respect to the vehicle group is observed, as well as a greater area of myelin and neurofilaments in the spinal cord. Furthermore, this molecule seems to have a direct effect on the biology of oligodendrocytes precursors cells (OPCs) by promoting their proliferation and differentiation towards myelinating phenotypes. This effect combined with other possible neuroprotectors could be a highly innovative strategy that would cover all aspects of the ideal therapy for MS.

T12-041D

Human 3D iPSC-derived brain model to study chemical-induced myelin disruption

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Human *in vitro* models to study myelin are very limited. Although myelin present an essential function in the nervous system and impairment of this membrane has been linked to many neurodegenerative diseases and neurological disorders. Thus, models and tools to study this relevant process are important to develop. We have previously developed a reproducible human iPSC-derived 3D brain model (also called BrainSpheres) that contains a high percentage of wrapping myelin for an *in vitro* model. Here, we have further developed this technology by applying different readouts to study myelination disruption. As proof of principle, we have used the BrainSpheres to assess different compounds potential to induce developmental neurotoxicity. The developing brain is susceptible to toxic insults, and there is concern that environmental chemicals contribute to widespread subclinical developmental neurotoxicity (DNT) such as autism and ADHD. Increased DNT evaluation is needed due to the lack of such

information for most chemicals in common use, but *in vivo* studies recommended in regulatory guidelines are expensive, time-consuming and difficult to interpret. Therefore, there is an ongoing effort to develop an in vitro testing battery covering different key processes of the developing brain to possible replace current in vivo guidelines. Myelination is one of the fundamental processes in neurodevelopment that should be included in such a DNT testing strategy. Thus, there is a need to establish an *in vitro* myelination assay for DNT. Here we identified compounds for assay development to evaluate the relevance of our BrainSphere model. Myelin was assessed by quantifying co-localization of myelin basic protein and neurofilament in confocal images of BrainSpheres, analysis of myelin-related gene expression, and quantification of other oligodendrocyte markers. In addition, early effects on astrocytes were investigated. Results demonstrated that the positive reference compound (cuprizone) and two of the three potential myelin disruptors (BPA, MetHq and TDCPP) caused reductions in myelination in the model, while ibuprofen (negative control) did not induce these changes.

Acknowledgement

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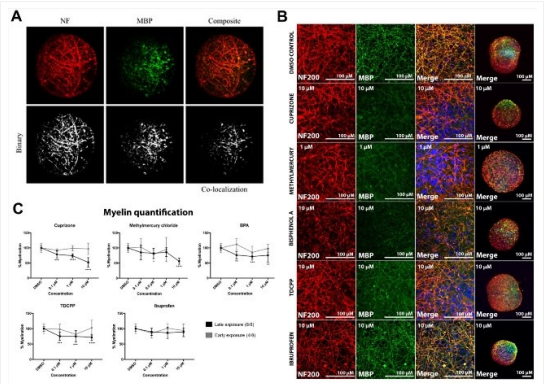
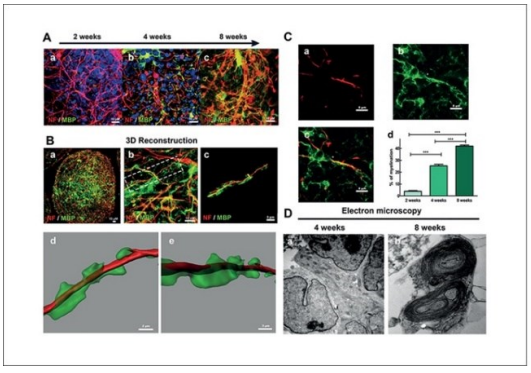


Figure 2. Chemical exposure alters myelin in BS. Chemical exposure alters myelin in BS. A) represent an example of binary conversion of confocal images using CEM plugin.B) shows confocal imagines of a representative BrainSphere after the higher non-cytotoxic concentration of each chemical. Treatment, concentration, and antibody used are indicated in each picture. White bars correspond to 500 µm. C) shows results from the BrainSphere myelination assay in which the percentage of myelination was quantified as co-localization of MBP and NF immunocytochemical staining in confocal images.



Myelin in BrainSpheres

(A) Co-immunostaining of neurons (NF) and the myelin marker MBP at 2, 4 and 8 weeks of differentiation (a, b, c, respectively) showed progressive increase of MBP+ cells in association with axonal processes. (B) 3D-reconstruction based on confocal z-stacks at 8 weeks. (C) MBP+ oligodendrocytes (green) issued processes in close association with axons (red) and seemed to enwrap them at 8 weeks (a, b, c). Myelination calculated as the mean percentage MBP positive oligodendrocyte processes coverage of NF-positive axons (a, b, c) at 2, 4 and 8 weeks (D) Electron microscopy analysis of BS

T12-042D

White matter matters in Gba1 pathologies

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Mutations in the glucocerebrosidase 1 (*GBA1*) gene do cause the lysosomal storage Gaucher's Disease (GD) but are also the most common genetic risk factor for Parkinson's Disease (PD). The mechanistic bases linking *GBA1* mutations to PD pathogenesis are not fully understood yet. Several studies suggested that a common mechanism shared by GD and PD is glucosylceramide accumulation within lysosomes, due to GBA1 dysfunction, thus leading to autophagy impairment and brain accumulation of autophagic substrates and α-synuclein aggregates. So far, studies on both PD and neuronopathic GD were primarily focused on neuronal manifestations, besides the evaluation of microglial and astrocyte implication. Instead, the contribution of oligodendrocytes (OCs) has been scarcely investigated. Of note, white matter alterations were suggested to sustain or even play a role in the PD process and were specifically described in PD patients with *GBA1* mutations. Moreover, transgenic mice overexpressing a-synuclein under OC-specific promoters develop neurodegeneration with parkinsonism outcomes, modelling multiple system atrophy (MSA), a still poorly studied synucleinopathy, characterized by α-synuclein aggregates primarily in OCs, in combination with neurodegenerative features.

In this context, by exploiting both *in vivo* and *in vitro* systems, we studied the role of Gba1 in myelinating glia and how this could be relevant in the onset of neurodegeneration. By crossing a line expressing a Cnp1-driven cre recombinase with mice carrying the *Gba1* catalytic site flanked by loxP sequences (*Gba1fl/fl*), we produced a conditional knockout mouse model for Gba1 in myelinating glia. Recombination and Gba1 enzyme inactivation were confirmed on myelinated tissues. The extent of central myelination was evaluated in control and conditional knockout mice, revealing a dysmyelination accompanied by neurodegenerative hallmarks. In parallel, *in vitro* analyses were performed in undifferentiated and differentiated oli-neu cells treated with conduritol B epoxide (CBE), a Gba1 irreversible inhibitor. CBE-induced inhibition was confirmed by the presence of reduced enzymatic activity and GlcCer accumulation, especially on differentiated oli-neu. Gba1 inactivation inhibited oligodendrocyte differentiation in vitro, by inducing lysosomal dysfunction and lipid dyshomeostasis. Our studies for the first time reveal a relevance for Gba1 in myelinating glia, showing that its inhibition in oligodendrocytes affects the lysosomal machinery and myelin protein expression, resulting in myelination deficits. This emphasizes the potential contribution from altered myelinating glia to the onset of GD and PD-related neurodegenerative features.

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T12-043D

Investigating the role of CNS myelination in circuit function and behaviour using zebrafish

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Myelin, formed by oligodendrocytes in the central nervous system (CNS), facilitates saltatory conduction of action potentials and provides trophic support to the wrapped axons underneath. Historically thought to be an inert structure, it is now clear that neuronal activity can alter the pattern of myelination along axons. Such adaptive myelination is expected to have palpable effects not only at the individual neuron level, but across neural networks, for example altering the timing of action potential arrivals at synapses. Indeed modulating myelination has been shown to affect behaviour. For example, preventing the formation of new myelinating oligodendrocytes has been demonstrated to impair certain aspects of rodent motor activity and memory. Furthermore, demyelinating disorders, such as Multiple Sclerosis, have devastating effects on the normal nervous system function with limited recovery. However, integrating how the regulation of myelination affects neuronal, synaptic, network and organism properties has been challenging to address in vivo.

In our study we looked at a zebrafish demyelination model, resulting in a disrupted CNS myelination profile, which is followed by rapid remyelination. To complement our findings, we also utilised a CNS-specific hypomyelination model. In this poster, we will present preliminary data on what effects demyelination, hypomyelination and remyelination have in intact larval zebrafish.

With our new multi-photon integrated setup we are able to perform live imaging to visualise both neuronal activation (by analysing calcium indicator activity) as well as behavioural outputs in response to physiological stimuli in wild-type, demyelinated, hypomyelinated and remyelinated zebrafish. The incorporation of an electrophysiological rig onto the system also allows us to patch-clamp neurons of interest and evaluate their physiological and synaptic properties. Such a multilevel platform approach therefore will enable us to bridge findings at cellular, network and organism levels in order to address what role demyelination, hypomyelination and remyelination have in vivo and how pharmacological interventions stimulating neuronal activity can alleviate demyelinating disease symptoms.

T12-044D

Aβ oligomers upregulate myelin basic protein mRNA transport and local translation in oligodendrocytes

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Myelin degeneration and white matter loss resulting from oligodendrocyte death are early events in Alzheimer's disease (AD) that lead to cognitive deficits. However, the underlying molecular mechanisms remain unknown. One of the hallmarks of AD is the presence of extracellular aggregates of amyloid beta peptide (Aβ), and Aβ oligomers (oAβ) have been proposed to induce changes in oligodendrocytes and myelin. Myelin basic protein (MBP), one of the most abundant proteins in the CNS myelin, is upregulated by oAβ. Nevertheless, Aβ-induced temporal and spatial control of MBP mRNA translation is still unclear.

Upon association with a subset of heterogeneous nuclear ribonucleoproteins (hnRNPs), MBP mRNA is sorted into granules and transported in an inactive state to the periphery of oligodendrocytes. Granule activation occurs upon phosphorylation of hnRNPs, which triggers local translation. Here, we characterized how oAβ control and deregulate the metabolism of MBP mRNA and its local translation in cultured oligodendrocytes. First, we observed that oAβ increase mRNA and protein levels of MBP, as well as of hnRNP A2, which binds to the 3´UTR of MBP mRNA and participates in its cytoplasmic transport. RNA immunoprecipitation revealed that oAβ treatment increase the levels of the MBP mRNA associated with hnRNP A2 in oligodendrocytes. In addition, analysis of functional association of hnRNP A2 with hnRNP F and hnRNP K to initiate mRNA MBP translation showed that oAβ increase the triple colocalization of ribonucleoproteins in distal domains, suggesting an increased proportion of active granules in oAβ treated cells. Moreover, phosphorylation levels of tyrosine residues of hnRNP A2 and direct visualization of newly synthesized MBP protein by Puro-PLA assay confirmed that oAβ increase the local translation of MBP in oligodendrocyte processes. Finally, consistent with *in vitro* results, we showed that oAβ promote overexpression of hnRNPA2 in mouse hippocampus after intracerebral injection of oAβ and in the hippocampus of 3xTG-AD mouse model, where MBP is also upregulated.

Taken together, these results suggest that oAβ alter the transport machinery of MBP mRNA and its localization, facilitating mechanisms for its local translation. This newly described process may influence the dynamics of oligodendroglial maturation and myelin sheath growth/extension, with potential consequences to electrical conduction.

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T12-045D

Deletion of monocarboxylate transporter 2 in oligodendrocytes leads todemyelination with oligodendroglial preservation

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Monocarboxylates (ketone bodies, pyruvate, and lactate) are alternative energy fuels that can be used by cells either constitutively, in addition to glucose, or under specific circumstances (hypoglycemia, etc). It is known that ketone bodies represent an important energy source for the brain during lactation, and lactate has been shown as a preferred energy substrate for neurons to maintain synaptic activity. Monocarboxylate trafficking into/outside the cells is achieved through monocarboxylate transporters (MCTs). While initial studies of MCT expression in the CNS showed that MCT2, a high affinity monocarboxylate transporter, is the predominant neuronal MCT, more recent studies of gene expression showed that this transporter is also expressed by microglia and cells of oligodendroglial lineage. Here, we have investigated MCT2 expression on oligodendroglial cells in the adult CNS. RNA sequencing data showed increased MCT2 expression in oligodendroglial cells with age. Protein expression of MCT2 was detected in the brain and spinal cord oligodendrocytes at different ages. To investigate the function of MCT2 in oligodendrocytes, we injected a Cre-GFP- or GFP-expressing oligodendrotropic AAV in the spinal cord white matter of wildtype (wt) or MCT2lox/lox mice. MCT2lox/lox mice injected with the Cre vector showed myelin loss and alterations associated with a mild increase in inflammation, without death of transduced oligodendrocytes. Instead, recombined oligodendrocytes showed a decrease in the expression of enzymes associated with lipid synthesis. Our results suggest that MCT2 expression in oligodendrocytes plays a role in lipid synthesis, likely by mediating monocarboxylate import. Yet, given that AAV injection per se leads to a slight increase in inflammation, it remains to be determined whether MCT2 is required for oligodendroglial lipid synthesis only under inflammatory conditions (that may alter the availability of metabolites), or also under basal conditions.

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T12-046D

Functional Consequence of Adult Onset Sulfatide Depletion in Mature CNS

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3-O-Sulfogalactosylceramide (sulfatide) is a sphingo-lipid that constitutes up to 4% of total myelin lipids in the central nervous system. To determine the function of this prominent myelin lipid, our lab ultrastructurally

characterized a mouse with a constitutively disrupted cerebroside sulfotransferase (CST) gene. CST catalyzes the final step in the production of sulfatide. Consequentially, the constitutive CST "knock-out" mice are incapable of synthesizing sulfatide. Using this mouse, our lab has shown that sulfatide is required for proper establishment and maintenance of myelin and the axoglial junctions that attach the myelin sheath to the axon and that provide stability to the nodal domains. In addition, we reported that sulfatide is involved in oligodendrocyte differentiation, and proliferation and that sulfatide may play a role in protein compartmentalization within the oligodendrocyte and myelin sheath. Interestingly, some of the ultrastructural pathologies that we reported in the CST KO mice are consistent with structural abnormalities observed in Multiple Sclerosis (MS). Moreover, sulfatide has been reported to be dramatically reduced in regions of normal appearing white matter (NAWM) of MS patients. Reduction of this lipid in regions of NAWM suggests that sulfatide depletion is independent of demyelination and is consistent with sulfatide depletion as a driving force of disease pathology and not merely a consequence of disease pro-gression. However, since MS is typically diagnosed in young adults, the constitutive CST KO mouse has limited clinical relevance since these mice develop in the absence of the lipid. In order to generate a more clinically relevant model, our lab has generated a "floxed" CST mouse, which provides both temporal and cell specific ablation of the CST gene. Using this mouse, mated against the PLP-creERT mouse we are investigating the structural and functional consequence that adult onset sulfatide depletion has on myelin sheath and axonal domain integrity and on oligodendrocyte function. Our preliminary data suggests that sulfatide depletion in the adult CNS causes nodal pathology and subsequent functional deficits to myelinated axons.

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T12-047D

NLRP3: a novel player in OPC proliferation and myelin regeneration

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Multiple Sclerosis (MS) is an immune-mediated demyelinating disease affecting about 2.5 million people worldwide. MS is characterised by a progressive loss of myelin and by the death of oligodendrocytes in a process called demyelination, which can ultimately lead to degeneration of neurons. In early stages of MS, neurodegeneration can be prevented by regeneration of myelin (remyelination). Although the molecular mechanism underlying this process is poorly known, it has been demonstrated that inflammation is required for efficient remyelination. Inflammation associated proteins, and in particular pro-inflammatory interleukins such as interleukin-1β (IL-1β) are abundant in plasma and CNS tissue of MS patients. The active form of IL-1β is released by inflammasomes, innate immune danger sensor complexes composed of a sensor protein (e.g. NLRP3), an adaptor protein (ASC) and caspase-1, the enzyme that cleaves pro- IL-1β and pro-IL-18 into their active forms. Given the high expression of IL-1β as well as proteins forming inflammasome complexes such as NLRP3 both in MS patients and murine models of MS, this study aims to understand whether NLRP3 is involved in remyelination of CNS lesions. Using a lysolecithin-induced focal demyelination model, we are able to study the different stages required for myelin regeneration.
Our preliminary data indicate that inflammasome components are present within demyelinated CNS lesions from early stages of remyelination suggesting involvement in this regenerative process. Using mice deficient in the inflammasome sensor NLRP3 we observe less proliferating oligodendrocyte progenitor cells (OPC) and more differentiated oligodendrocytes within CNS lesions at early stages of the repair process. We also investigated other glial cells involved in damage repair such as astrocytes and microglia/macrophages. At early stages of remyelination, NLRP3 deficiency has no effect on astrocytes whereas an increased number of microglia/macrophages was present within the lesion area.
All together, these data suggest that NLRP3 may have an active role in regulating OPC proliferation and/or differentiation after demyelination, which could affect the remyelination process. In addition, the effect of NLRP3 on microglia/macrophages may also have an impact on the repair process, as these cells are required for efficient remyelination. Further analyses into the underlying mechanisms will help to define the role of NLRP3 and the inflammasome complex in remyelination and might uncover molecular targets to promote myelin repair.

T12-048E

TRPA1 regulates potassium syphoning and seizure susceptibility through modulation of oligodendrocyte potassium channels

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Oligodendrocytes are highly specialized glial cells characterized by their production of multilayer myelin sheaths that wrap axons to speed up action potential propagation. It is due to their specific role in supporting axons that impairment of myelin structure and function leads to a wide range of neuronal dysfunctions such as neuronal hyperactivity, conduction block and epileptic seizures. Glial cells take part in K^+ buffering by removing excess ions that accumulate in the periaxonal space of myelinated axons via inward rectifier K^+ (K_{ir}) channels. For this purpose, Kir4.1 is highly expressed by oligodendrocytes. Its activation is ATP-dependent, and its loss leads to epileptic

seizures. Through the presence of Kir4.1, the extracellular [K^+] balance is smoothly restored, and a new action potential can be easily generated. Myelin loss can be receptor-mediated and recently oligodendrocytes have been shown to express the non-selective cation channel TRPA1, activation of which raises oligodendrocyte $[Ca^{2+}]_i$ resulting in myelin damage in ischaemia.

Here, we show that TRPA1 activation can inhibit around 45% of the resting K^+ conductance in oligodendrocytes, and this is attenuated by high concentrations of intracellular ATP, as well as by blocking phosphatases. Blocking $K_{ir}4.1$ with the selective inhibitor VU 0134992 reduces the resting K^+ conductance of oligodendrocytes by ~30%. However, subsequent activation of TRPA1 then inhibits a further 60% of the K^+ conductance. These results suggest that TRPA1 activation inhibits potassium channels by reducing ATP levels but the involvement of Kir4.1 needs to be investigated further. Importantly, mice lacking TRPA1 channels exhibit a similar phenotype to Kir4.1 knockout, including having depolarised oligodendrocyte resting membrane potentials and a lower seizure threshold, possibly due to an impairment in K^+ buffering.These results support our earlier suggestion that TRPA1 inhibition could be protective in demyelinating diseases, but also suggest a new function for TRPA1 in regulating the excitability of neurons by controlling K^+ syphoning through the glial network.

T12-049E

Reduced myelination and altered Schwann cell adaptation to injury in a mouse model lacking GBA1 in myelinating glia

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Lysosomal glucocerebrosidase (GBA1) activity is pivotal in cells which either have a high lipid catabolism or need an efficient autophagic clearance capacity. *GBA1* mutations affecting its folding, transport or enzymatic activity cause a lysosomal storage disorder called Gaucher disease (GD). Furthermore, *GBA1* mutations are risk factors for early onset Parkinson's disease, as the linked lysosomal defects have a role in alpha-synuclein aggregation and spread in the brain. Medical reports point at a possible involvement of peripheral nerves in GD with small fiber neuropathy, neuropathic pain and parkinsonism. Schwann cells (SCs), make up the lipid-rich myelin sheaths of peripheral nerves (PN), provide trophic support to axons and insulate them almost entirely from the outer environment. In case of irreversible injury to axons, denervated SCs undergo a process of reprogramming, inducing autophagy and structural rearrangements to support both axon degeneration and regeneration. Autophagic clearance of myelin and axonal debris has a key role in Wallerian degeneration, as myelin can inhibit axonal regrowth. With the aim of establishing whether lysosomal glucocerebrosidase expressed by SCs is important for myelination in basal conditions and for both myelin degradation and re-myelination of peripheral nerves upon injury, we characterized the peripheral phenotype of a conditional KO (cKO) mouse model for GBA1 in myelinating glia. GBA1 expression in sciatic nerves (SN) increases through post-natal development up to the 6 months of age. At 6 months of age myelin defects are detectable, in terms of reduced levels of myelin-related proteins analyzed by western blotting and immunofluorescence, as well as reduced myelin-relevant lipids as observed by Fluoromyelin staining. Myelin alterations, supported by TEM images, were parallelled by hallmarks of altered SC maturation and inflammation, indicating a role for GBA1 in myelination during development. To study the involvement of SCs GBA1 in nerve degeneration and regeneration we also adopted the unilateral sciatic nerve crush model. cKO mice displayed a delay in myelin degradation at early stages and in SFI recovery at 14 dpi.

Nevertheless, at 21 dpi cKO mice achieved a complete recovery, comparably to controls. These results support the presence of a defect in early degradation of myelin by GBA1 defective SCs, then carried out by macrophages recruited to the nerve. Further data will elucidate whether inefficient lysosomal activity has a role in the onset or development of peripheral neuropathies caused by demyelination, given the importance of endosomal trafficking and recycling in myelin maintenance.

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T12-050E

Experience-dependent myelination following stress is mediated by the neuropeptide dynorphin

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Emerging evidence implicates experience-dependent myelination in learning and memory. However, the specific signals underlying this process remain unresolved. We demonstrate that the neuropeptide dynorphin, which is released from neurons upon high levels of activity, promotes experience-dependent myelination. Following forced swim stress, an experience that induces striatal dynorphin release, we observe increased striatal oligodendrocyte precursor cell (OPC) differentiation and myelination, which is abolished by deleting dynorphin or blocking its endogenous receptor, kappa opioid receptor (KOR). We find dynorphin also promotes developmental OPC differentiation and myelination, and demonstrate that this effect requires KOR expression specifically on OPCs. We characterize dynorphin-expressing neurons and use genetic sparse-labeling to trace their axonal projections. Surprisingly, we find they are unmyelinated normally and following forced swim stress. We propose a new model whereby experience-dependent and developmental myelination is mediated by unmyelinated, neuropeptide-expressing neurons that promote OPC differentiation for the myelination of neighboring axons.

T12-051E

Inefficient demyelination can compromise axonal integrity in models of toxic demyelination

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Multiple sclerosis, an autoimmune disorder affecting more than 2 million people worldwide, is characterized by demyelinating lesions in the central nervous system. Importantly, neuronal loss rather than demyelination itself is the main contributor to long-term neurological symptoms. However, the actual implication of demyelination to axonal pathology and degeneration remains only partially understood. We here applied two models of demyelination, lysolecithin and cuprizone, in order to analyze axonal integrity as a function of the myelination state in the central nervous system white matter. Our data indicate that inefficient demyelination, in contrast to rapid and efficient demyelination, contributes to irreversible axonal damage, possibly downstream of impaired axonal support by the perturbed myelinating oligodendrocyte. In detail, upon lysolecithin-induced demyelination, a large numbers of swollen axons, characterized by organelle accumulation, arise. In contrast to the rapidly demyelinating lesion center, we found an increased number of swollen axons to be still myelinated at the lesion border where demyelination kinetics are decelerated. Interestingly, in this area, we also noticed a higher number of irreversibly degenerated axons which were almost exclusively myelinated. In line, also in the cuprizone model, axons with irreversible signs of damage were found to be myelinated throughout the time course of demyelination. In addition, we noticed numerous axonal swelling at the time of ongoing demyelination. Interestingly, these axons completely vanished when full demyelination was completed, while axonal numbers remained unchanged. We hypothesize that rapid demyelination might be beneficial for the short-term survival of axons while retarded or incomplete demyelination may pose axons at special risk to degeneration. These data highlight the complexity of myelin-axon-interactions and the need to better understand involved mechanisms in demyelinating diseases.

T12-052E

Extracellular HMGB1 inhibits oligodendrocyte progenitor cell differentiation via TLR2 signaling

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HMGB1 is a highly conserved, ubiquitous protein found in nearly all eukaryotic cells. When released, extracellular HMGB1 becomes an alarmin, an extracellular class of proteins that have functions in the unstressed cell, but are released by stressed cells to act as a danger associated molecular pattern (DAMP). HMGB1 is normally localized to the nucleus, where it acts as a chromatin associated non-histone binding protein. Extracellular HMGB1 has been found to act as a mediator of sterile inflammation as well as inflammation associated with many conditions. We have recently determined that progenitor cells from multiple sclerosis patients develop a cellular senescence phenotype and release HMGB1 (Nicaise et al. 2019 *PNAS* 116; 9030-9038). This finding is in line with recent studies detailing significantly increased levels of extracellular HMGB1 in MS patient plasma (Bucova et al. 2019, *Neurol Sci* 3;599-604). We had also determined that HMGB1 directly impairs the maturation of OPCs to myelinating oligodendrocytes (OLs). Herein, we report on the receptor-mediated mechanism through which HMGB1 affects OPCs. HMGB1 is known to initiate and propagate stress and inflammation induced signaling through the toll-like receptors (TLRs), or the receptor for advanced glycation end products (RAGE). To define the receptor for extracellular HMGB1 that mediates its inhibition of OL differentiation, we examined which of these receptors were expressed by primary rat OPCs and determined that all four putative receptors were expressed. To test which of these receptors was responsible for HMGB1 signaling, we tested selective inhibitors of TLR2,4, 9 or RAGE in the presence of HMGB1. From this we determined that inhibition of TLR9 or RAGE did not rescue OL differentiation, while inhibition of TLR4 resulted in partial restoration of OL differentiation and inhibiting TLR2 resulted in a complete block of HMGB1s effects. To validate this, we determined the concentration-dependent effect of TLR2

inhibition on HMGB1 inhibition of OL differentiation. We also determined that HMGB1 activation of TLR2 on OPCs resulted in nuclear translocation of NFκB in OPCs, in a rapid, concentration dependent manner. We then examined RNAseq data from HMGB1-treated OPCs to define the downstream transcriptional response and focused on NFκBs regulated gene targets. These data provide new information on how extracellular HMGB1 impairs the differentiation potential of OPCs. Because HMGB1 is expressed in demyelinated lesions in MS, greater understanding of how this molecule impairs OPCs differentiation may represent a strategy to promote endogenous remyelination in MS.

Acknowledgement

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T12-053E

Investigating the role of CCN3 in central nervous system myelination and remyelination

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Multiple sclerosis is a demyelinating disease of the central nervous system (CNS) characterised by immune-mediated destruction of myelin-producing oligodendrocytes. While there is a plethora of disease-modifying therapies to reduce relapses, there are currently no available treatments to boost oligodendrocyte and myelin regeneration. Regulatory T cells secrete CCN3, a matricellular protein that promotes oligodendrocyte progenitor cell (OPC) differentiation and myelination in murine brain slice cultures. These findings identified CCN3 as a candidate of interest in CNS myelination and therefore we sought to investigate the expression and role of CCN3 in myelin development and regeneration.

Oligodendrocytes and myelinated axons were quantified in CNS white matter of wild type (WT) and CCN3-deficient (KO) mice. We found no difference in the number of oligodendrocytes or myelinated axons between groups, suggesting that CCN3 is not required for CNS myelination. To investigate the role of CCN3 in OPC differentiation during remyelination, WT and CCN3 KO mice were injected with lysolecithin in the ventral white matter of the spinal cord, creating a focal demyelinated lesion. There was no difference in the number of proliferating OPCs at 5 days post lesion (dpl), or differentiated oligodendrocytes at 14 dpl in WT and KO groups. In a model of cuprizone-induced demyelination, there was no significant difference in the number of oligodendroglia or differentiated oligodendrocytes in the corpus callosum during remyelination in WT and CCN3 KO mice. Overall, these results suggest that CCN3 is not required for OPC proliferation or differentiation in the CNS. However, interestingly CCN3 was transiently upregulated in the brain and spinal cord during demyelination and remyelination respectively. Furthermore, around 40% of CCN3-expressing cells in the remyelinating spinal cord were oligodendroglia. These results suggest that despite robust and dynamic expression, CCN3 is not required for myelination or remyelination in the mouse CNS.

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T12-055E

The role of TRPA1 in regulating action potential amplitude in the mouse optic nerve

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Introduction: Oligodendrocytes wrap myelin sheaths around axons in the CNS thereby insulating them and facilitating energetically efficient propagation of action potentials along the neuron [1]. The importance of myelin is demonstrated in injury such as ischaemic stroke where myelin damage is attributed to the high influx of Ca^{2+} into oligodendrocytes [1]. TRPA1 is a non-selective cation channel expressed on oligodendrocytes but its role in glial cell functioning is unknown [1]. We hypothesised that TRPA1 channels on oligodendrocytes regulate action potential amplitude and cause loss of action potential propagation during excessive channel activation.

Methods: In a blinded study, the role of TRPA1 in the optic nerve was assessed using compound action potential (CAP) recordings. Optic nerves from young adult C57Bl6J mice were dissected and the effects of TRPA1 agonists and antagonists on the CAP amplitude were evaluated. TRPA1 antagonist, A967079, was applied acutely (20μM) or preincubated (10μM) before recording. The effect of the CAP amplitude in the presence of A967079 and TRPA1 agonist, polygodial (10 or 100μM), were investigated, along with the effect of high (4 mM) and low (30 μM) extracellular Ca^{2+}concentrations. Statistical significance was determined using a Student's T-test or One- Way ANOVA with Holm- Bonferroni multiple comparison test ($P<0.05$).

Results: A967079 alone produced a 20% increase in CAP amplitude once applied to the mouse optic nerve. In the absence of A967079, polygodial produced a decrease in CAP amplitude in a concentration-dependent manner: 6% (10μM) and 30% (100μM) decrease. A967079 reduced the decrease in CAP amplitude produced by polygodial compared to vehicle control to 0% (10μM) and 20% (100μM) ($P<0.01$). Interestingly, external solution containing 30μM Ca^{2+} produced a 15% increase and 4mM Ca^{2+} produced a 10% decrease in CAP amplitude, which recovered upon application of normal solution.

Conclusion: These results suggest that TRPA1 is tonically active and supresses the CAP in the mouse optic nerve and that further activation of TRPA1 with polygodial causes an A967079 sensitive decrease in CAP amplitude. To investigate whether the changing flux of Ca^{2+} may play a role in this phenomenon, we altered extracellular Ca^{2+} concentrations. The changes caused mimic the effect of the CAP amplitude seen with TRPA1 agonists/antagonists. These results indicate that TRPA1 has a role in regulating action potential amplitude. TRPA1 antagonism has neuroprotective properties in conditions mimicking ischaemia, by reducing myelin damage [1]. This suggests that TRPA1 channels could be a therapeutic target in demyelinating disease and injury. Future studies will further investigate the role of TRPA1 in glial cell functioning.

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T12-057E

Striatin-3 is a novel Rac1 interactor in Schwann cells

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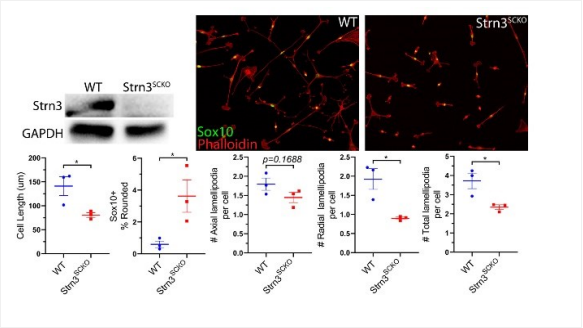
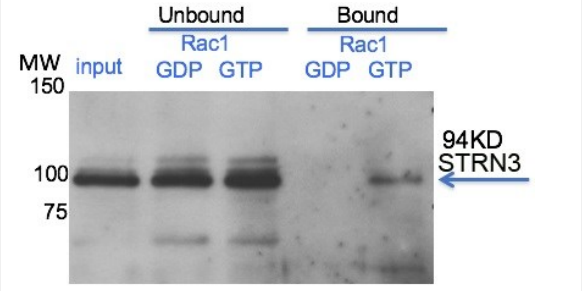
During development, Schwann cells (SCs) undergo extensive cytoskeletal reorganization as they insert cytoplasmic extensions into axon bundles to sort, ensheath, and myelinate individual axons. Similarly, following peripheral nerve injury there is extensive actin polymerization around Schmidt-Lantermann incisures (SLIs) as Schwann cells differentiate into a repair phenotype. Both of these processes are regulated by Rac1. Our laboratory previously demonstrated that Rac1 activation in SCs is driven by engagement of α6β1 integrin with laminins, and that this is essential for peripheral nerve development (Nodari et al., 2007). We then performed a proteomic screen to look for novel Rac1 interactors in peripheral nerves and identified striatin-3 (Strn3) as a candidate. Strn family proteins (Strn1/3/4) function as the core scaffolding proteins of STRIPAK (STRiatin-Interacting Phosphatase And Kinase) complexes. STRIPAK complex members include the phosphatase PP2A and the upstream Hippo pathway kinases Mst1/2. PP2A dephosphorylates Mst1/2 to deactivate the Hippo pathway, permitting nuclear translocation of the transcriptional co-activators Yap/Taz. Our group previously demonstrated that Yap/Taz activity in SCs is critical for peripheral nerve development (Poitelon et. al., 2016). Knockdown of Strn3 in primary rat SCs decreases their ability to adhere to various substrates, reduces proliferation, and disrupts their association with axons. Using Strn3 floxed mice expressing P0-Cre, we have specifically ablated Strn3 in SCs ($Strn3^{SCKO}$). Sciatic nerves of $Strn3^{SCKO}$ mice demonstrate mild radial sorting defects and hypomyelination. Strn3 null SCs isolated from these animals and plated on laminin have impaired cell elongation, process extension, and lamellipodia formation, similar to SCs deficient in Rac1. Preliminary evidence suggests that the loss of Strn3 *in vivo* is partially compensated for by upregulation of Strn1 and Strn4. Therefore, we have generated double knockout mutants ($Strn1/3^{dSCKO}$ and $Strn3/4^{dSCKO}$) to investigate potential compensatory mechanisms. Additionally, our work will investigate mechanisms linking the STRIPAK complex with Rac1 and the Hippo pathway in SCs. This work seeks to define the role of Strn3 and the STRIPAK complex in SC development and injury response.



Strn3 null Schwann cells exhibit Rac1-like defects. Schwann cells were isolated from WT and Strn3^{SCKO} mice and plated on laminin. Similar to Schwann cells with ablation of Rac1, the Strn3 null Schwann cells exhibit defects in cell elongation and lamellipodia formation.



Strn3 preferentially interacts with active Rac1. Sciatic nerve lysates of post-natal day (P5) wild-type mice were exposed to Rac1-bound beads pre-charged with either GDP (inactive) or GTP (active). Rac1 preferentially pulls down Strn3 when in its active, GTP-bound state.

T12-058E

What is the Role of Actin Disassembly in CNS Myelination?

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In the central nervous system (CNS), oligodendrocytes extend numerous processes to ensheath and spirally wrap axons with myelin. Myelin enables fast and efficient nerve conduction, gives axons metabolic support, and provides activity-induced plasticity in the CNS. Myelin loss and the inability to remyelinate are a hallmark of many neurological disorders, including multiple sclerosis, yet the cellular mechanism that drives myelin wrapping is largely unknown. Myelin wrapping was thought to be powered by actin filaments at the distal tip of an oligodendrocyte (the inner tongue) similar to an axonal growth cone. Surprisingly, previous studies found that actin filaments are disassembled during myelination and suggest alternate hypotheses for the forces that drive myelin wrapping: (1) Actin disassembly could “trigger” actin- myelin wrapping with no need for further actin filament assembly, or (2) Repeating cycles of actin disassembly and reassembly could “ratchet” the myelin membrane forward during wrapping. To test between these models, we first set to determine whether actin filaments are

present during wrapping. We defined myelination stages in a specific spinal cord region, the thoracic gracile fasciculus, to study actin dynamics at different myelination stages. We found that in this region, ensheathment of axons begins at P4, myelin wrapping takes off at P8 and continues beyond p24. Using high-resolution light microscopy in the same region, we found that actin filaments are abundant in myelin rings during ensheathment, but later disappear as wrapping begins. Next, we induced actin disassembly in oligodendrocytes *in vivo* during wrapping by injecting AAV packaged constructs to express DeActs (genetically encoded actin disassembly tools we invented) driven by the MBP promoter that becomes highly active in myelinating oligodendrocytes. Injection of pMBP-DeAct constructs effectively induced loss of actin filaments in oligodendrocytes without affecting ensheathment. Incredibly, inducing oligodendrocyte actin disassembly with DeActs led to the formation of thicker myelin. Together, our results suggest that actin disassembly serves to initiate wrapping and that wrapping does not require sustained actin filament assembly, consistent with the “trigger” model of myelin wrapping. Our future work is focused on understanding the actin-independent forces that drive myelin wrapping, identifying signals regulating actin disassembly, and testing whether this mechanism is perturbed in demyelinating diseases.

T13 | Neural stem/progenitor cells

T13-001C

Lineage relationships of the derived-cell progeny of Neural Progenitor Cells

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Neural Progenitor Cells (NPCs) are generated from a pool of neuroepithelial cells that undergo symmetric divisions to produce Radial Glial Cells (RGC). These cells, in partially overlapped waves, first produce a number of self-amplifying divisions and then became to be asymmetric, generating neurons, astrocytes, NG2-glia and oligodendrocytes. Few embryonic cortical progenitors appear to be lineage-multipotent, suggesting a progenitor diversity more restricted to one specific lineage. Other studies show a potential cell diversity depending on the spatio-temporal pattern, reinforcing the idea that **NPCs constitute a heterogeneous population**. Besides, NG2-glia is another remarkable cell type that can act as a progenitor. Although we know how NPCs produce the diverse cell progeny, many unknowns about lineage relationships, progenitor subtypes, and their heterogeneity remains unravelled.

To deciphering the cell potential of NPCs, it is necessary to analyze the derived-cell progeny of single GFAPor NG2-progenitors targeted with stable and heritable labelling. To target single NG2 or GFAP-progenitor cells in pallial SVZ we used **UbC-(NG2-PB)-StarTrack** or **UbC-(GFAP-PB)-StarTrack**, respectively. After *in utero* electroporation, at different mouse embryonic stages (E12, E14 and E16), with these modifications of UbC-StarTrack, we performed a clonal analysis of the derived-cell progeny at P30. Our findings provide fundamental aspects of the lineage cell potential and heterogeneity of NPCs during development.

Acknowledgement

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T13-002C

Wnt/β-catenin signaling promotes neurogenesis in the ischemic adult mouse brain

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Canonical Wnt signaling orchestrates the fate of neural stem cells (NSCs) during embryogenesis; however, its role in proliferation/differentiation of postnatal neural stem/progenitor cells (NS/PCs) under ischemic conditions remains elusive. To shed light on this, we employed transgenic mice, modulating Wnt signaling upon tamoxifen-induced cre-mediated DNA recombination. Therefore, we were able to manipulate Wnt signaling at three different subcellular levels – inhibit it at the membrane (expression of Wnt blocker Dickkopf 1) or in the nucleus (expression of dominant negative form of T-cell factor), or hyper-activate it via production of a stable variant of β-catenin, the principal element of the pathway. In addition, we hypothesized that the modulation of the Wnt signaling pathway in the adult mouse brain may enhance endogenous regenerative processes and thus compensate negative consequences of ischemic injury. To disclose the impact of Wnt signaling inhibition/activation on NS/PCs derived from the subventricular zone (SVZ) of adult mice following ischemic injury, we employed middle cerebral artery occlusion, a model of permanent focal cerebral ischemia (FCI). First, we analyzed NS/PCs differentiated *in vitro* utilizing immunostaining against β-catenin and found that activation of Wnt signaling resulted in higher expression of this protein, while inhibition of the pathway led to the opposite effect. Next, we assessed membrane properties of differentiated cells and revealed that cultured NS/PCs gave rise to three distinct cell populations – astrocytes, neuron-like cells, and precursors. Surprisingly, our functional analysis *in vitro* showed that their incidence was unaffected by Wnt signaling modulation under physiological conditions. Nevertheless, a higher abundance of neuroblasts marked by doublecortin, glial-fibrillary-acidic-protein-positive astrocytes, and dividing cells harboring proliferating cell nuclear antigen was detected in ischemic coronal brain sections, indicating an increased proliferation and differentiation rate of NS/PCs in the SVZ. Additionally, single-cell RNA sequencing revealed higher proportion of NSCs and neuroblasts following FCI. Importantly, Wnt signaling activation increased the numbers of proliferating and neuron-like cells, while its inhibition in the cell nucleus increased the counts of astrocytes. Consistent with these observations, our electrophysiological analysis revealed fewer neuron-like cells after Wnt signaling inhibition at the cell membrane level. Collectively, our results suggest that Wnt signaling supports neurogenic potential of postnatal NS/PCs. Interestingly, this effect is enhanced under ischemic conditions, which might be applied in NS/PCs-based regenerative therapy of the adult brain affected by stroke.

Acknowledgement
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T13-003C

Reproducing diabetic retinopathy features using newly developed human iPS-derived retinal Müller glial cells.

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Muller glial cells (MGCs), the glial cells of the retina, are responsible for the homeostatic and metabolic support of this tissue. Despite the importance of MGCs in retinal disorders, reliable and accessible human cell sources to be used to model MGC-associated diseases are lacking. We developed a protocol to generate and bank human induced pluripotent stem cell-derived MGCs (hiMGCs). Using a transcriptome analysis, we showed that the 3 genetically independent hiMGCs generated were homogeneous and showed phenotypic characteristics and transcriptomic profile of primary MGCs (pMGCs). These cells expressed key MGC markers, including Vimentin, CLU, DKK3, SOX9, SOX2, S100A16, ITGB1 and CD44 and could be cultured up to passage 8. Using a disease modelling approach, we showed that hiMGCs could be used to model the features of diabetic retinopathy (DR)-associated dyslipidaemia. First, palmitate, a major free fatty acid with elevated plasma levels in diabetic patients, induced the expression of inflammatory cytokines found in the ocular fluid of DR patients such as CXCL8 (IL-8) and ANGPTL4 and the analysis of palmitate-treated hiMGC secretome showed an upregulation of proangiogenic factors strongly related to DR, including ANG2, Endoglin, IL-1β, CXCL8, MMP-9, PDGF-AA and VEGF. Second we demonstrated an CXCL8 amplification loop when hiMHGCS are cultured in the presence of PA-stressed mononuclear phagocytes conditionned medium. Thus, hiMGCs could be an alternative to pMGCs and an extremely valuable tool to help to understand and model glial cell involvement in retinal disorders, including DR.

T13-004C

Cell type-specific vulnerability to traumatic brain injury in the hippocampal neural stem cell niche

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Traumatic brain injury (TBI) is a major global health concern arising from everyday life activities, such as domestic and road accidents, annually affecting 69 million people worldwide. Nearly 50% of TBI patients experience long term cognitive impairment linked to the dysfunction of the hippocampus. Immunohistochemistry experiments in

mouse hippocampus have shown that cortical TBI affects neural stem and progenitor cells (NSPCs) and induces severe astrogliosis.

Here we bring together fully complementary single cell RNA sequencing and highly multiplex *in situ* transcriptomics datasets (a novel *in situ* transcriptomics technology developed by Apollo Life Sciences GmbH) to characterize the impact of cortical TBI on NSPCs, immature astrocytes and immature neurons residing in the sub-granular layer of dendrite gyrus. We systematically assess the sensitivity of these cell types to TBI-induced pathology at the molecular level and provide the spatial map of the most variable genes at the single cell resolution.

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T13-005C

"Milking": an innovative approach to investigate the properties of postnatal brain neural stem cells and to obtain oligodendrocyte progenitor cells from live experimental rats

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Postnatal brain neural stem and progenitor cells (NSPCs) cluster in anatomically defined stem cell niches, one of which is the subependymal zone (SEZ) at the lateral walls of the lateral ventricles and are characterized by a wide differentiation potential, self-renewal and quiescence. Oligodendrocyte progenitor cells (OPCs) give rise to myelinating oligodendrocytes and also exhibit self-renewing potential. Here, we refine our method of "milking" the rat ventricular system in order to collect OPCs from the corpus callosum and to investigate the basic properties of SEZ-resident NSPCs. Milking consists of an intracerebroventricular injection of a release cocktail containing neuraminidase, β1-integrin blocking antibody and Fibroblast Growth Factor-2 in order to induce the controlled flow of NSPCs and OPCs in the cerebrospinal fluid. At a second "collection" step, liquid biopsies of CSF are performed from the cisterna magna of anesthetized experimental animals without the need of an incision. Liquid biopsies after milking caudal ventricular areas resulted in the isolation of cells expressing at high percentages typical OPC markers, such as Olig2 and PDGFRα. Furthermore, cells isolated after milking of the SEZ, that we have previously shown to exhibit characteristics of quiescent neural stem cells, were cultured in three different growth media. The typical NSPC medium, containing FGF2 and EGF and two media known to favor the expansion of neural stem cells without enhancing their progress towards differentiation. Our results showed significant differences in the morphology of grown cells and in their colony-formation characteristics that we investigate further using a range of NSPC markers in order to directly assess the profile of endogenous SEZ.

T13-007C

Role of Renin-Angiotensin System in the Regulation of Adult and Aged Rodent Ventricular-Subventricular Zone Neurogenesis

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The ventricular-subventricular zone (V-SVZ) lining the lateral ventricles is a well-known neurogenic niche whose adjacent location to the striatum points out the resident neural stem cells (NSCs) as a promising therapeutic tool to repair lesioned circuits in Parkinson's disease. NSCs express mature astroglia markers such as GFAP, GLAST and BLBP but they also exhibit an apico-basal polarity inherited from the embryonic radial glia. Their apical processes allow NSCs to contact cerebrospinal fluid in which a variety of regulatory factors are present. However, the regulatory network underlying neurogenesis and the possible factors linked to its decline with age remain poorly understood. The renin-angiotensin system (RAS) and particularly its angiotensin type 2 receptors (AT2) have been classically involved in processes of cell proliferation and maturation during development. Nevertheless, the possible role of RAS on neurogenesis remains unclear. In the present study, we analysed the role of main RAS receptors on neurogenesis in the V-SVZ of adult mice and rats. In mice, we showed that the increase of proliferation cells in the neurogenic niche was induced by the activation of AT2 receptors but partially relied on AT2-dependent antagonism of AT1 receptor expression. AT1 receptors, which exert pro-oxidative and pro-inflammatory actions in different tissues, constitutively restricted proliferation in the adult rodent V-SVZ. Furthermore, we observed a functional dependence of AT2 receptor actions on Mas receptors. Interestingly, in rats, where the levels of AT1 relative to those of AT2 receptor are much lower, pharmacological inhibition of AT1 receptors alone was sufficient in increasing AT2 receptor levels and proliferation in the V-SVZ. In aged rats, we observed a decrease both in proliferation and in neurogenesis which was reverted to young control levels by antagonizing AT1 activity. Our data revealed that interactions between RAS receptors play a pivotal role in the regulation of adult neurogenesis in the V-SVZ, particularly in proliferation, generation of neuroblasts and migration to the olfactory bulb, both in young and aged brains. Taken together, our results open the door to the possibility of new therapeutic approaches based on the use of RAS modulators to enhance adult neurogenesis.

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T13-008D

Acute loss of proliferating NG2$^+$ cells after spinal cord injury chronically alters scar formation and axon growth

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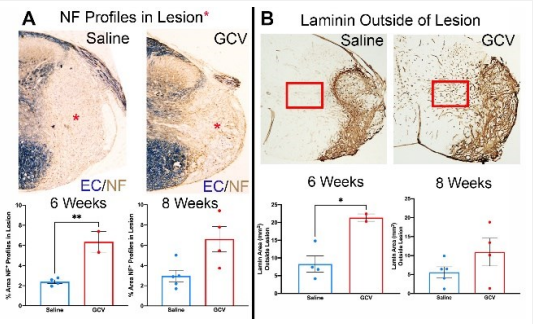
The extent to which NG2$^+$cells contribute to glial and fibrotic scar formation after spinal cord injury (SCI) is poorly understood. To selectively ablate dividing NG2$^+$cells responding to SCI, we utilized a novel transgenic mouse line in which cells expressing NG2 also express a thymidine kinase from herpes simplex virus (NG2-tk mice). Intraventricular administration of antiviral agent ganciclovier (GCV) causes apoptosis in dividing NG2$^+$ cells (including oligodendrocyte progenitors and pericytes). Immediately following unilateral white matter-specific C5 SCI in NG2-tk mice, a drug delivery pump was placed subcutaneously with the attached catheter inserted into the ventricle to administer GCV or saline for 14 days. Our prior work showed ablation of NG2$^+$ cells significantly altered the density and distribution of glial and fibrotic scars through 21 days post-injury. Compared to controls, these alterations prolonged hemorrhage, enhanced edema, and impaired forelimb recovery, but also increased axons entering the lesion area (Hesp et al., 2018). The present study assessed the long-term consequences of acute NG2$^+$cell ablation. GCV or saline were administered for the first 14 days after injury, then pumps were removed for a recovery period before assessment at six or eight weeks after SCI. In contrast to earlier time points, overall density of GFAP in the glial scar and laminin within the fibrotic scar did not significantly differ between NG2$^+$cell-ablated and non-ablated and mice. However, there were differences in scar density and patterning, suggesting altered recovery after GCV cessation (Fig. 1). Consistent with acute findings, significantly more axonal profiles were maintained in the lesions in GCV-treated mice at both 6w and 8w after SCI (Fig. 2A). A unique pattern emerged in the gray matter adjacent to the lesion, with a significant increase in laminin profiles resembling blood vessels in NG2$^+$ cell-ablated mice at both time points (Fig. 2B). This is currently being verified with additional markers and suggests NG2$^+$ cell ablation alters subsequent blood vessel structure in spared tissue after SCI. Impaired motor recovery detected acutely after SCI was maintained through 8w post-injury. Overall, these findings indicate acute ablation of proliferating NG2$^+$ cells after SCI chronically alters glial and fibrotic scars, axonal growth into the lesion, motor function and blood vessel structure in adjacent spared gray matter. Clarifying the role in these changes of NG2+ oligodendrocyte progenitors versus pericytes will be important to further define the role of each cell type in the beneficial and deleterious cellular responses to CNS injury.



NG2 cell ablation alters NF profiles within the lesion and laminin concentration outside the lesion

A. Initial results suggest even at late time points, more neurofillament (NF) profiles enter the SCI lesion site (red asterisk) in mice given ganciclovir (GCV) to ablate NG2$^+$cells acutely after injury than control mice recieving saline. **B.** Our preliminary findings reveal GCV administration increased laminin staining over saline controls in the gray matter adjacent to the SCI lesion (red box). This impliies blood vessles outside of the immediate lesion area are altered long-term in response to early NG2 cell ablation.

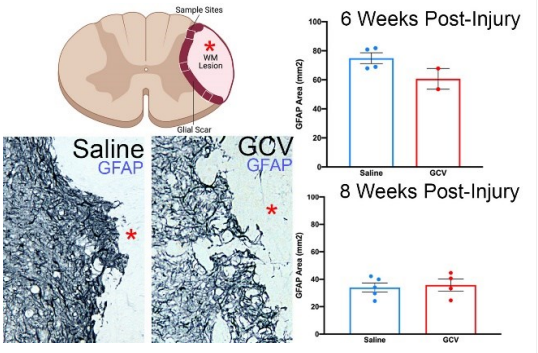


Figure 1: Long-term GFAP within the glial scar

Sample images (white boxes) were collected from within the glial scar, directly adjacent to the SCI lesion area (red asterisk). GFAP staining suggests early ablation of NG2$^+$cells through ganciclovir (GCV) administration altered scar density and patterning as compared to control mice recieving saline. Though findings are preliminary, the overall concentration of GFAP did not significantly differ between groups at 6 or 8 weeks post-injury. This is in contrast to earlier timepoints in which clear alterations in GFAP concentration were observed within the glial scar (Hesp et al., 2018).

T13-009D

Searching for modulators of adult oligodendrogenesis

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Oligodendrocytes (OLs) are the myelin-forming cells in the Central Nervous System of vertebrates. Disruptions in OLs or in the myelin sheath may result in demyelinating diseases, being Multiple Sclerosis (MS) the most common. Studies have shown that under demyelinating conditions, oligodendrocyte precursor cells (OPCs) present in the brain parenchyma can generate new OLs, which spontaneously remyelinate newly nude axons in damaged areas. Importantly, neural stem cells present in the subventricular zone (SVZ-NSCs) are a source of OLs that contribute to repopulate the lesioned regions, as it was shown in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS and in the cuprizone (CPZ) mouse model of demyelination. As disease progresses, the spontaneous reparative process that occurs in acute MS lesions fails, leading to axonal degeneration and neurological and cognitive deficits. Our group has studied the role of several modulators, such as brain-derived neurotrophic factor (BDNF), cannabinoid receptors type 1 and 2 (CBRs) and adenosine A2A receptors (A2ARs) on postnatal neurogenesis. Still, the role of these modulators on adult oligodendrogenesis from SVZ-NSCs remains unknown. Hence, we aimed at studying how these modulators and the putative crosstalk between adenosine A2ARs and CBRs/BDNF can influence OL differentiation from postnatal SVZ-NSCs. To do so, we used the neurosphere assay to generate SVZ-NSCs that were pharmacologically treated with adenosine A2ARs modulators, CBR ligands or BDNF. Cell differentiation into the oligodendroglial lineage was studied by immunocytochemistry for markers of OPCs (NG2/PDGFRα) and mature OLs (MBP). Results obtained so far show that treatment with BDNF tends to increase OPC formation after 4 days *in vitro* (DIV) (n=3; CTRL set to 100% and 203.8±27.59 for BDNF; $p=0.0548$), whilst significantly increasing the number of OPCs at DIV7 (n=3; CTRL set to 100% and 243.0±22.16 for BDNF; $p<0.05$), without affecting OL maturation. No changes were observed after treatment with A2ARs and CBR modulators. Ongoing work entails the study of cell proliferation by immunocytochemical colocalization of BrdU (a synthetic thymidine analogue used to study cell proliferation) with markers of the oligodendroglial lineage. Also, the cellular mechanisms behind the observed effects and changes in OL and myelin-related genes will be evaluated through western blot and RT-PCR, respectively. To date, this work outlined the role of BDNF in promoting the formation of OPCs derived from SVZ-NSCs, and with the ongoing work we expect to unravel a role for adenosine A2ARs, CBRs and BDNF in the modulation of adult oligodendrogenesis, thus contributing to the development of alternative therapeutic strategies for OL formation and remyelination.

T13-010D

Neurogenic and oligodendrogenic cell fate decisions of postnatal brain Neural Stem Cells are differentially dependent on their microenvironment

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Two major populations of stem/progenitor cells co-exist in the postnatal mammalian brain: multipotent Neural Stem Cells (NSCs) and the more lineage-restricted oligodendrocyte progenitor cells. These populations exhibit distinct spatial preferences, with the former being located only within specialized microenvironments, called stem cell niches, while the latter being broadly dispersed in the brain parenchyma. Here, we employ a cell culture assay in which postnatal brain NSCs were cultured as neurospheres and subsequently were plated on PDL-coated coverslips to create a range of microenvironments, that we classified either as “niche-like” 3D areas, or as “parenchyma-like” 2D areas. Cells were, then, induced to differentiate and the acquisition of a neurogenic or oligodendrogenic cell fate was correlated with the cytoarchitecture of their microenvironment. Our results demonstrated that neurogenesis is much more dependent on the architecture of surrounding cells, observed at significantly higher levels in the “parenchyma-like” areas, although often at the periphery of the “niche-like” structures, being proportional to the total cell density. On the other hand, oligodendrogenesis was found to be independent of the architecture and cell density of the microenvironment. In the presence of added laminin (a major extracellular component of the niche) cell cultures became more homogeneously two-dimensional, but neurogenesis was switched to a more “niche-like” behaviour, while oligodendrogenesis was not affected. Notably, the inhibition of β1 integrin -aiming at disrupting laminin-dependent signaling- further enhanced the diversion between the two cell fates, almost eliminating neurogenesis but increasing oligodendrogenesis. Finally, the administration of BNN-20, a microneurotrophin previously shown to increase NSC differentiation, was the only factor that affected both cell fates in the same direction, leading to significant increases. In conclusion, our novel method of analysis revealed that neurogenesis exhibits higher microenvironment restrictions than oligodendrogenesis and constitutes a new tool for the investigation of the effects of possible therapeutic strategies in specific properties of neurogenesis and oligodendrogenesis.

Acknowledgement

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T13-011D

Loss of Jedi-1 impairs microglial phagocytosis, resulting in reduced postnatal neurogenesis in the subventricular zone.

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Jedi-1 is an engulfment receptor that mediates phagocytic clearance of apoptotic sensory neurons by satellite glia in the developing murine peripheral nervous system. The clearance of apoptotic debris is also critical for the development and maintenance of the central nervous system, in particular for postnatal neurogenesis.

Neurogenesis relies on the coupling of neural precursor proliferation, newborn neuron apoptosis, and microglial clearance of the apoptotic debris. Using immunofluorescent labeling in brain sections from male and female wildtype (WT) and *Jedi-1* knockout mice (JKO) in the first week of postnatal life, we show that Jedi-1 is expressed in WT microglia residing in the postnatal neurogenic niche, the ventricular/subventricular zone (V/SVZ), but absent in the knockout. Therefore, we asked whether Jedi-1 expression in microglia contributes to this coupling and, thereby regulates neurogenesis. To test whether loss of Jedi-1 hinders microglial phagocytic ability, we employed an *in vitro* engulfment assay and find that JKO microglia display a significant reduction in engulfment relative to WT microglia. This finding is recapitulated by an accumulation of apoptotic cells in the JKO V/SVZ, as shown by TUNEL assay. To determine whether loss of Jedi-1 and subsequent disruption of microglial phagocytic ability impacts neural precursor proliferation, we performed an EdU pulse at postnatal day 7 *in vivo*. Our findings demonstrate that JKO mice have fewer proliferating neural progenitors in the V/SVZ relative to WT mice. Furthermore, JKO mice have reduced numbers of MASH1^{+} newborn neurons when compared to those of WT mice. Together, these data support the hypothesis that postnatal neurogenesis is maintained in part by Jedi-1-dependent microglial phagocytosis of apoptotic newborn neurons.

T13-012D

Unveiling the heterogeneity of vertebrate adult Neural Stem Cells

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The vertebrate brain harbors neural stem cells (NSCs) that persist into adulthood to ensure the life-long production of functional neurons. These NSCs are mainly quiescent, a feature important for their maintenance into old age. The adult zebrafish pallium hosts NSCs with similar characteristics to their mammalian counterparts but present in large number, making it a powerful model to unravel NSC properties. Using this model, our lab recently demonstrated the existence of subpopulations of NSCs that differ in their quiescence depths[1] and/or are hierarchically organized along a cascade leading to activation and neuronal production[2]. However, the molecular signature and functionally relevant markers for these subpopulations remain unknown. In order to get a better description of the heterogeneity of quiescent NSCs we used single-cell RNA-sequencing with the 10x Genomics platform. We recovered over 17k cells, including more than 3k quiescent NSCs, making it the most extensive dataset on the adult zebrafish forebrain and on adult NSCs to date. This allowed us to discover new cell subtypes, and in particular highlighted the existence of previously undescribed glial sub-clusters that differ in their level of quiescence and/or commitment. To uncover functional interactions between clusters and identify potential master regulators of NSC quiescence, we developed and adapted tools applied to scRNA-seq analysis in mammals for use with zebrafish. We then re-analyzed datasets generated from murine NSCs[3,4] in order to confront our data with theirs, allowing us to generate lists of putative conserved regulators that may play a role in controlling quiescence depth.

Finally, we repeated this scRNA-seq analysis on zebrafish pallial NSCs upon transient blockade of Notch signaling, which poises NSCs for activation. This further revealed the existence of a rare population of NSCs resistant to the activation by Notch blockade, and identified a putative regulator of their quiescence and expression of E(spl)

genes. These results together provide molecular signatures for NSC heterogeneities, including for a novel, Notch-independent, deeply quiescent NSC sub-population.

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T13-013D

Single cell multi-omics of the adult neural stem cell lineage reveals coordinated changes in DNA-methylation, chromatin accessibility and gene expression

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The ventricular-subventricular zone is one of few stem cell niches in adult mammalian brains, harboring a population of adult neural stem cells (NSCs) which generate both glia and neurons. The advent of single cell RNA-sequencing made it possible to study transcriptional changes along the adult NSC lineage at unprecedented resolution, but it is still unclear whether epigenetic mechanisms such as DNA-methylation (DNA-m) control the observed expression changes and fate decisions. Several studies demonstrated a crucial role for DNA-m in embryonic NSC function, however the role of DNA-m in adult NSCs is currently unknown since they are too rare to assess DNA-m with conventional methods.

Here, we used single cell multi-omics as well as low-input whole genome bisulfite sequencing (WGBS) to identify and characterize DNA-m changes in the adult NSC lineage *in vivo*, and to assess how they relate to transcriptional changes. Using WGBS, we identified thousands of differentially methylated regions (DMRs) between freshly isolated NSCs, neuroblasts (NBs) and oligodendrocytes.

We hypothesized that DNA-m of these genomic regions is one factor that controls cell identity. In line with this hypothesis, we found that DMRs are located near cell type-specific genes such as myelin production genes in oligodendrocytes. Surprisingly, we rarely observed DNA-m changes in promoters, but rather in promoter flanking regions and enhancers.

Very recently, single cell multi-omics methods emerged as a promising tool to study whether gene expression change is accompanied by epigenetic change. To this end, we employed scNMT-seq, a novel multi-omics method that enables simultaneous profiling of gene expression, DNA-m and chromatin accessibility of single cells. We made several improvements to the protocol. Most notably, we increased the throughput from 96 cells to 384 cells per experiment, added unique molecular identifiers (UMIs) to eliminate PCR-bias, and developed a computational approach to identify genomic regions with variable DNA-m in single cell data.

scNMT-seq data of adult NSCs and their progeny confirmed the DMRs previously identified in WGBS and furthermore showed that they occur largely independent of changes in chromatin accessibility. Due to the single

cell resolution of these data, we were able to predict potential target genes of DMRs by correlating gene expression with DNA-m. Surprisingly, we found that changes in DNA-m are a good predictor of gene expression changes in NSCs and oligodendrocytes, but not in NBs, suggesting that other gene regulatory mechanisms are involved.
Our study provides a detailed view of the epigenomic and transcriptomic landscape of the adult NSC lineage. We conclude that DNA-m and transcription occur in a coordinated manner.

T13-014D

Interferon signaling shapes neural stem cell differentiation dynamics

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Neural stem cells (NSC) within the adult subventricular zone (SVZ) and the subgranular zone in the dentate gyrus of the hippocampus continuously generate neurons that promote flexible learning and adaptive behavioural responses to cognitive and emotional challenges. We have previously shown that depletion of adult NSCs in the ageing brain is prevented through the tight regulation of quiescence by inflammatory cytokines. Specifically type I IFN response increases with age in the whole brain. However, the time point at which interferons become crucial for regulation of stem cell dynamics is still elusive. Here, we show that upon deletion of interferon receptor alpha and gamma (IFNA/G-R KO), NSC from the SVZ migrate promptly to the striatum and differentiate into striatal neurons. As a consequence, the number of NSCs and transit-amplifying progenitors (TAP) in the SVZ was found to be lower in IFN KO mice compared to WT. By single-cell transcriptomic profiling of adult-mice NSC we found that in active NSC and TAPs, the negative regulator of neurogenesis Dynl1b is downregulated upon IFN KO, suggesting a positive role of IFN in this process.
To define quantitative aspects of neurogenic lineage progression, we applied a mathematical model of SVZ neurogenesis to quantifications of WT and IFNKO stem cell populations. We found that the initially reduced number of NSCs in the KO can be explained by a constant rate of activation. Surprisingly, we found that older IFN KO mice are still able to sustain a population of NSCs. Our modelling suggests that this is due to an increase of self-renewal in ageing.
By time-lapse microscopy at the single-cell level we demonstrate that activated NSC from IFNKO mice undergo faster exhaustion, with a corresponding increase in apoptosis. Finally, we addressed the functional consequences of IFN signalling loss by behavioural studies. At the neonatal stage IFNKO mice show an abnormal pattern of ultrasonic vocalizations, which indicates impaired embryonal neurogenesis. Moreover, IFNKO mice display impaired social novelty behaviour, which is not caused by a decrease in olfactory bulb neurogenesis. These mice also present an impaired memory consolidation capacity, which correlates with a decreased number of NSC in the dentate gyrus of the hippocampus. Altogether, these findings advance our understanding of the role of IFN signaling in NSC homeostasis.

T13-015E

Spatial and temporal recruitment of adult neural stem cells during pregnancy

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Neurogenesis in the adult rodent brain is restricted to few highly specialized niches. The largest site of adult neurogenesis is the ventricular-subventricular zone, which extends along the brain ventricles. V-SVZ neural stem cells (NSCs) give rise to different subtypes of olfactory bulb interneurons as well as glia throughout life. NSCs are not a uniform population. Depending on their location in the V-SVZ and their molecular identities they show distinct differentiation fates. The balance between quiescent and activated stem cells is tightly controlled by the niche, but also by extrinsic and long-range signaling from other brain areas. These signals are still largely unknown. Here, we show that pregnancy activates specific stem cell subpopulations residing in spatially distinct domains of the V-SVZ, with different temporal dynamics. The recruitment of 'pregnancy-related' NSCs is transient, and results in the generation of both common and rare subtypes of olfactory bulb interneurons that mature at physiologically relevant time points in motherhood. Interestingly, oligodendrocyte progenitors also increased in the olfactory bulb concomitant with the increase in neurons. Taken together, our results suggest that 'on-demand' neurogenesis and gliogenesis occurs during pregnancy, eventually contributing to crucial aspects of maternal behaviour.

T13-016E

Stem cell-derived intraventricular oligodendrocyte progenitors in the adult brain

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Quiescent neural stem cells (NSCs) in the adult ventricular-subventricular zone (V-SVZ), next to the lateral ventricles, undergo activation to generate neurons that migrate to the olfactory bulb and some glia. V-SVZ NSCs have a regional identity and give rise to different subtypes of interneurons, depending on the spatial domain in which they reside. However the domains for gliogenesis are less explored. We have found that Platelet-Derived Growth Factor Receptor beta (PDGFRβ) is expressed by adult V-SVZ NSCs. Selective deletion of PDGFRβ in adult V-SVZ NSCs leads to their release from quiescence and revealed multiple gliogenic domains and cell types. Unexpectedly, we identified a novel intraventricular oligodendrocyte progenitor derived from NSCs inside the brain ventricles. Intraventricular OPCs were also present in wild type brains, localized on top of ependymal cells. Using lineage tracing and immunostaining, we show that intraventricular OPCs are derived from $GFAP^+$ NSCs, and exhibit morphological and molecular differences compared to adult parenchymal OPCs. Interestingly, intraventricular OPCs first appeared around postnatal day 5, paralleling an increase in PDGFAA ligand in the cerebrospinal fluid. Notably many intraventricular OPCs were closely apposed to or partially enwrapped supraependymal serotonergic axons present on the surface of both the lateral and septal walls. Given their location

inside the ventricles, intraventricular OPCs are uniquely poised to integrate and dynamically respond to signals from the cerebrospinal fluid and diverse cell types, including axons from other brain regions.

T13-017E

Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion

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In the adult brain, subsets of astrocytes act as neural stem cells in two anatomically defined neurogenic niches: the sub-ventricular zone and hippocampal dentate gyrus. Surprisingly, after excitotoxic lesion striatal astrocytes acquire stem cell properties and generate a large amount of neuroblasts for at least six months. Yet the presence and organization of striatal neurogenic niches and the spatio-temporal dynamics of striatal astrocytes activation and lineage progression remain by large unclear.

Here, through genetic lineage-tracing experiments and 3D reconstructions coupled with mathematical modelling and computer simulations we dissected the transition of striatal astrocytes toward neurogenesis. In the striatum, neurogenic astrocytes are scattered throughout the parenchyma and expand locally, generating clusters of clonally related cells, that we define as striatal niches. These structures are initially composed only of activated astrocytes and transient amplifying progenitors. These latter cells subsequently expand and generate proliferating neuroblasts following a stochastic mode of division and differentiation. Post-mitotic neuroblasts accumulate in the cluster before dispersing as individual cells. Interestingly, striatal astrocytes become activated at a constant rate, resulting in the continuous addition of new striatal niches with time. Nevertheless, the total number of niches does not increase with time indicating that these structures have a transient existence. Thus, continuous striatal neurogenesis occurs through the asynchronous transition of scattered neurogenic astrocytes from quiescence to an active state.

Overall, these data suggest that the neurogenic potential is widespread among striatal astrocytes, and that the striatal parenchyma is largely permissive for de-novo establishment of neurogenic niches.

T13-018E

A neural stem cell nich with an embryonic-like dorsal-ventral regionalization conserved in the aged human spinal cord

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Anamniotes and rodents maintain neural multipotent cells in the ependymal zone (EZ) around the central canal of the spinal cord. Our previous RNA profiling showed that immature developmental genes are still expressed even in the young human EZ. These ependymal cells maintain an embryonic-like spinal cord organization with the expression of typical spinal cord developmental/stem cell transcription factors such as Arx, Msx1, Pax6 or Sox2, 4,6,11 and cilia transcription factor FoxJ1. We and others found that these cells are multipotent and can generate neurons and glial cells in vitro. The maintenance of these cells in the adult or aged human spinal cord is still debated. We addressed this pending issue by collecting fresh spinal cords from 10 ageing humans (from 53 to 83 y.). Using immunolabelling techniques, we found a persistent lifelong expression of spinal cord developmental factors (Arx, Pax6, Msx1, Sox2 and FoxJ1) in the human EZ. A dorsal-ventral regionalization and a central lumen are also observed in most cases at all ages. The persistence of these embryonic-like cells in the aged human spinal cord potentially represents an interesting endogenous cellular source to alleviate various spinal cord lesions.

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T13-019E

Neurotoxic effects of three environmental toxicants exposure using *in vitro* model of murine neurospheres.

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Context: Environmental pollutants, like glyphosate (GLY) and ammonium glufosinate (GLA) are herbicids with high persistence in environment. Because of their huge utilization, they widely participate to eutrophication which leads, in association with climate change, to cyanobacterial blooms.β-methylamino-L-alanine (L-BMAA) is a cyanotoxin suspected to be a neurotoxic. This toxicant could induce long-term learning alterations and memory deficits but this remains unclear. Indeed, the genetic causes of neurodegeneratives diseases are well known but the environmental causes are probably underestimated because studies are often far from the real conditions of exposure (exposome). Early exposure to environmental contaminants are known to induce stress and/or inflammatory responses. This can permanently alter brain development and thus confer predispositions to develop various neuropsychiatric and/or neurodegenerative pathologies. *In vitro* neural stem cells (NSC) cultures can provide an alternative for studying adverse effects of environmental contaminants on critical neurodevelopmental processes, including cell proliferation and differentiation.
Goal: Our purpose is to determine the ability of cocktail exposure (GLY, GLA, BMAA) to induce neurotoxic effects and/or neuroinflammation, using a 3D neurospheroids models.
Method: NSC were isolated from ventricular-subventricular zone from offspring from PND0 to PND5. NSC and microglial cells are then cultivated in 2D before 3D-culture on 96-well array plates. Our 3D neurospheroids model have the capacity to mimic neurogenic niches due to their ability to self-organized and to allow communication between different cell populations. In order to elucidate if cocktail exposure impairs NSC differentiation and/or neuroinflammation, neurospheres cultures were treated with 3µM of GLA, 30µM of GLY and 100µM of BMAA during astroglials differentiation.
Results: Our results shows that astrocytes and microglial population are impacted by the combination of three environmental toxicant. By flow cytometry, we notified a decrease of astrocytes population and proliferation of microglials cells. Those results were also observed at gene expression level. Considering that microglial cells play

a key role in CNS development including cell differentiation we suspect impact on gene expression for the others glials cells types present in our model. Moreover, our preliminary datas suggest that the cocktail exposure may induce a neuroinflammation mediated through NF-κB and STAT3 pathways.

T13-020E

Regenerative neurogenic response from glia requires insulin driven neuron-glia communication

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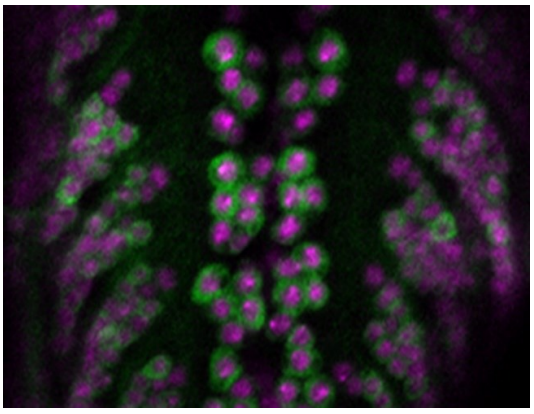
Understanding how injury to the Central Nervous System (CNS) induces de novo neurogenesis in animals would help promote regeneration in humans. Regenerative neurogenesis could originate from glia and glial Neuron-Glia antigen-2 (NG2) may sense injury-induced neuronal signals, but these are unknown. Here, we used *Drosophila*to search for genes functionally related the *NG2*homologue *kon-tiki (kon),*and identified *Islet Antigen-2 (Ia-2),*required in neurons for insulin secretion. Alterations in Ia-2 function induced neural stem cell gene expression, injury increased *ia-2*expression and induced ectopic neural stem cells. Using genetic analysis and lineage tracing, we demonstrate that Ia-2 and Kon regulate*Drosophila*insulin-like peptide 6 (Dilp-6), to induce glial proliferation and neural stem cells from glia. Ectopic neural stem cells can divide, and limited de novo neurogenesis could be traced back to glial cells. Altogether, Ia-2 and Dilp-6 drive a neuron-glia relay that restores glia, and reprograms glia into neural stem cells for regeneration.



Ia-2 is exclusively neuronal.
Transmembrane Ia-2YFP surrounding Elav+ neuronal nuclei. Image by Elizabeth Connolly (Harrison, Connolly et al 2021 eLife).

T13-021E

Interferon type I modulates stemness and differentiation of adult Neural Stem Cells via cell cycle control and protein synthesis regulation

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The ventricular-subventricular zone (V-SVZ) is the largest germinal center of the mammalian adult brain populated with neural stem cells (NSCs). We discovered that an upregulation of a number of interferon-activated genes in NSCs upon ageing is responsible for the age-related decline in neurogenesis in the V-SVZ[1]. Interferons modulate the stemness and differentiation potential of different adult stem cells in mammals, albeit invoking different effects in a tissue-dependent manner[2,3].

In NSCs, the presence of interferon type I halts cell cycle progression and regulates protein synthesis in a timely manner. Ribosomal profiling showed that interferon type I upregulated the translation of Interferon-Stimulated Genes (ISGs) while reducing global protein translation in NSCs *in-vitro*. The control of protein translation was time-dependent showing an upregulation and later downregulation in short and maintained exposure to interferon type I, respectively. We show that such regulation occurs in a mTORC1-dependent manner and that ISGs and a subset of other genes can escape the later downregulation.

Interestingly, on the onset of differentiation, NSCs precisely modulate post-transcriptionally genes involved in the maintenance of stemness and the progression of neurogenesis[4]. Among those, a group of genes containing a pyrimidine-rich motif (PRM), including Sox2, is found now downregulated also upon long exposure to interferon type I. In addition, the integration of ribosomal profiling and proteomics of different datasets suggests that NSCs treated with interferon type I *in-vitro* resemble both more quiescent and committed states, simultaneously.

Altogether, our study identifies molecular targets governing the response of NSCs upon inflammation. and how the inflammatory cytokine interferon type I modulate the stemness and differentiation of NSCs via cell cycle control and stemness-related mRNA post-transcriptional regulation.

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T14 | Neuroimmunology and neuroinflammation

T14-001A

Region-dependent changes in microglial dynamics in grey matter in a cuprizone model of demyelination

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Microglia are key players in Multiple Sclerosis (MS), expressing many susceptibility genes for this disease. They constantly survey the brain microenvironment, but the precise relationship between microglial dynamics and pathological processes in MS remains unknown. We used a model of chronic MS, induced in mice by dietary cuprizone, a copper chelator that causes oxidative stress, mitochondrial dysfunction, and selective loss of oligodendrocytes, resulting in extensive demyelination of both white and grey matter in the brain. As in MS, cuprizone-treated mice show early and extensive microglial activation in both white and grey matter. However, histopathology used for identifying microglial modifications has had limited spatial and temporal resolutions and was mainly focused on pathology in white matter areas. Using high-resolution confocal and 2-photon imaging and a newly developed approach for analysing individual microglial cell dynamics, MicroApp, we found that heterogeneity in microglial morphology and function is disease and region-dependent and is associated with differences in demyelination and remyelination. In particular, we found that in cortical layer 5 and hippocampal CA1 microglia became activated very early in response to cuprizone, before detectable demyelination and showed region-specific characteristics. In cortical layer 5 microglia formed nodules with increased phagocytic activity while in CA1 they changed morphology becoming less ramified and more hypertrophic. Demyelination had similar region-specificities, starting earlier in cortical layer 5 but being more complete in hippocampal CA1. In contrast, in cortical layer 2/3, microglia were not significantly involved in phagocytosis and demyelination, but instead showed changes during remyelination, becoming hyper-ramified with slower process movement, thereby maintaining similar local tissue surveillance properties. Thus, profiling of microglial activation using specific morphological, functional and motility parameters may be useful as a sensitive biomarker for disease progression in the grey matter in MS.

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T14-002A

Diet-dependent regulation of TGFb impairs reparative innate immune responses after demyelination

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Pro-regenerative responses are required for the restoration of nervous system functionality in demyelinating diseases such as multiple sclerosis (MS). Yet, the limiting factors responsible for poor CNS repair are only partially understood. Here, we test the impact of Western diet (WD) on phagocyte function in a mouse model of demyelinating injury that requires microglial innate immune function for a regenerative response to occur. We find that WD feeding triggers an ageing-related, dysfunctional metabolic response that is associated with impaired myelin debris clearance in microglia, thereby impairing lesion recovery after demyelination. Mechanistically, we detect enhanced transforming growth factor beta (TGFb) signalling, which suppresses the activation of the liver X receptor (LXR)-regulated genes involved in cholesterol efflux, thereby inhibiting phagocytic clearance of myelin and cholesterol. Blocking TGFb or promoting triggering receptor expressed on myeloid cells 2 (TREM2) activity restores microglia responsiveness and myelin debris clearance after demyelinating injury. Thus, we have identified a druggable microglial immune checkpoint mechanism regulating the microglial response to injury that promotes remyelination.

T14-003A

Heterogeneity of microglial nodules in MS: possible implications for MS lesion formation

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Background: Microglia are phagocytic cells of the brain involved in multiple sclerosis (MS) pathology. In MS, microglia can form small cell clusters, known as nodules, which may initiate lesion formation. However, microglial nodules are found also after stroke. Here we characterized nodules in MS and stroke tissue to assess their possible contribution to MS lesion formation.
Methods: To identify disease-specific traits, we characterized nodules in post-mortem normal-appearing white matter stroke (n=4) and MS (n=4) tissue by immunohistochemistry for MS lesion-enriched proteins. In addition, we determined the number, size, and proliferation state of nodules as well as the nearby presence and characteristics of lymphocytes. Furthermore, we analyzed the relation between nodules and clinical and neuropathological parameters in 121 MS brain donors.
Results: In MS tissue, the number of HLA-DR$^+$ nodules was higher as compared to stroke tissue, and the majority of MS nodules contained proliferating microglia. Microglia within nodules in stroke and MS expressed proteins implicated in phagocytosis (MSR1 and CD11c), lipid accumulation (CHIT1), or T-cell regulation (CD86), but the frequency of nodules expressing these molecules was higher in MS. Interestingly, only in MS, proliferating lymphocytes were present in the parenchyma near some nodules, and expression of genes for antibody production was only found in MS nodule tissue. Additionally, in 121 MS donors, cases with nodules had a higher load of reactive sites and white matter lesions and a higher proportion of active lesions, as compared to donors without nodules, suggesting an association between the presence of nodules and the initiation of MS lesions.
Conclusions: Altogether, molecular and cellular patterns as well as clinical associations suggest a role for nodules in MS lesion formation.

T14-004A

Major histocompatibility complex-expressing oligodendrocyte lineage cells are induced in mouse models of multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune, demyelinating disease affecting more than 2 million people worldwide. While disease-modifying therapies for MS are currently available, there are no treatments that directly augment remyelination, the failure of which is central to disease pathophysiology. Remyelination is thought to be mediated by the differentiation of oligodendrocyte precursor cells (OPCs) and/or regeneration of new sheaths by existing oligodendrocytes. Recently, our lab and others have shown that the inflammatory cytokine interferon gamma induces expression of major histocompatibility complex (MHC) class I and II molecules in OPCs. These “inflammatory OPCs” (iOPCs) can present antigens to CD4+ and CD8+ T cells *in vitro*, potentially modifying remyelination. However, the extent of iOPC induction and the spatial and temporal dynamics of this process over the course of disease remain unknown.
To answer these questions, we engineered MHC class I and class II reporter mice in which a P2A-tdTomato (tdT) sequence replaced the stop codon of beta-2 microglobulin (B2M) or cluster of differentiation 74 (CD74),

respectively. Expression of tdT in these *B2M-P2A-tdT* and *CD74-P2A-tdT* reporter mice was restricted to cells immunoreactive for MHC class I and II, respectively. In *CD74-P2A-tdT* control mice, tdT expression was only observed outside the central nervous system, and in *B2M-P2A-tdT* control mice, tdT was observed in blood vessels and microglia/macrophages of the brain and spinal cord, consistent with the known expression patterns of MHC class I and II. We examined tdT expression in two animal models of MS: actively induced experimental autoimmune encephalomyelitis (EAE) and adoptive transfer of myelin-specific CD4+ T cells into mice previously demyelinated with cuprizone (AT-CPZ). In both models, tdT+ (MHC-expressing) Olig2+ oligodendrocyte lineage cells were observed in the brain and spinal cord, with tdT+/Olig2+ cells more prevalent in *B2M-P2A-tdT* mice. The AT-CPZ model induced tdT expression in Olig2+ cells at higher levels than in EAE in both reporter lines. Moreover, the abundance of tdT+/Olig2+ cells was associated with disease severity in both EAE and AT-CPZ, with more iOPCs observed in advanced stages of disease and in regions with increased inflammation. The relationship between MHC-expressing Olig2+ cells and clinical disease in demyelinating mouse models support a role for iOPCs in immune-mediated demyelinating diseases such as MS, where they may modulate inflammation and cellular reorganization. The ability to track iOPCs during disease using these reporter mice will help reveal how MHC expression in oligodendrocyte lineage cells modifies disease progression and expand our knowledge of the roles of OPCs in heath and disease.

T14-005A

Spermidine ameliorates neuroinflammation and Alzheimer's disease pathology

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Alzheimer´s disease (AD), the most common cause of dementia, is not only characterized by extracellular amyloid plaque formation and neurofibrillary tangles deposition, but also by microglia- and astrocyte-mediated neuroinflammation. In the last decade, impairment of autophagy has been found to be yet another important feature of AD linking neuroinflammation and Aβ pathology.

The nutritional supplement Spermidine is a known autophagy activator which mediates anti-inflammatory effects in the context of multiple sclerosis in the experimental autoimmune encephalomyelitis mouse model (Yang et al. 2016, Cell Death Diff). Here, we investigated Spermidine´s therapeutic potential in an AD-like mouse model. Oral Spermidine treatment of APPPS1 mice resulted in decreased neuroinflammation and a reduction of neurotoxic soluble Aβ at both early and late stages of pathology. *In vitro* analyses revealed how Spermidine reduced cytokine release in adult and neonatal microglia as well as neonatal astrocytes. In line with previous findings, we found that Spermidine mediated its anti-inflammatory effects in an autophagy-dependent manner underlining that autophagy is a crucial mechanism for controlling neuroinflammation.

Based on the various effector functions of Spermidine in glial cells, Spermidine appears to be an attractive supplement to attenuate neuroinflammation and AD.

T14-006A

Sustained hyperammonemia induces TNF-a in glial cells and Purkinje neurons by activating the TNFR1-NF-κB pathway

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Patients with liver cirrhosis may develop minimal hepatic encephalopathy which reduces quality of life and life span. Rats with chronic hyperammonemia reproduce the cognitive and motor alterations, which are mediated by peripheral inflammation and neuroinflammation with activation of microglia and astrocytes. Motor incoordination is due to increased TNF-a and activation of its receptor TNFR1 in cerebellum. It remains unclear how hyperammonemia increases TNF-a in cerebellum and in which cell types.

The initial aims were to assess in hyperammonemic rats: a) if peripheral inflammation is responsible for induction of TNF-a b) in which cell type(s) is TNF-a increased.

We analyzed the content of TNF-a and of its mRNA in microglia, astrocytes and Purkinje neurons in cerebellum at 4 weeks of hyperammonemia and assessed if preventing peripheral inflammation by injecting anti-TNF-a prevents TNF-a induction. At 4 weeks of hyperammonemia, TNF-a expression was induced in microglia, astrocytes and Purkinje neurons and this was prevented by treatment with anti-TNF-a.

We then aimed: c) to provide some insights on the mechanisms: to assess whether 1) TNF-a induction is associated with increased nuclear NF-κB; 2) if this is mediated by TNFR1 activation, by testing ex vivo in cerebellar slices if TNF-a induction is reversed by R7050, which inhibits the TNFR1-NF-κB pathway; d) To analyze the time-course of TNF-a induction in glial cells and Purkinje neurons

Sustained (4 weeks), but not short-term (2 weeks) hyperammonemia induces TNF-a in Purkinje neurons in rats. Hyperammonemia induces microglia and astrocytes activation at 1 week. This is followed by TNF-a induction in both glial cell types at 2 weeks and in Purkinje neurons at 4 weeks. The amount of mRNA for TNF-a increases in parallel with TNF-a protein, indicating that it is synthesized into Purkinje cells. This increase is associated with increased NF-κB nuclear translocation. Nuclear translocation of NF-κB and the increase of TNF-a are reversed by R7050, indicating that are mediated by activation of TNFR1. The results support that hyperammonemia induces TNF-a in glial cells and TNF-a released by glial cells would activate TNFR1 in Purkinje neurons leading to NF-κB nuclear translocation and induction of TNF-a expression which may contribute to the neurological alterations in hyperammonemia and hepatic encephalopathy by altering the Purkinje neurons function and neurotransmission.

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T14-007A

Time-resolved single-cell RNAseq profiling identifies a novel *Fabp5*-expressing subpopulation of inflammatory myeloid cells in chronic spinal cord injury

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Traumatic spinal cord injury (SCI) is a highly debilitating pathology that devastates thousands of individuals annually. This pathology is characterised by an initial mechanical insult, followed by a highly dynamic and a spatiotemporally complex secondary injury. A key player in the evolution of the secondary injury is inflammation, with infiltrating bone marrow-derived macrophages (BMDMs) and resident microglia (MG) as primary drivers. Here we use time-resolved single-cell RNA sequencing (scRNAseq) to deconvolute the complex heterogeneity of BMDMs and MG in mouse models of SCI. We then validate and elaborate upon this *ex vivo/in silico* data via small-molecule fluorescent *in situ* hybridization (smFISH), immunohistochemistry, and *in vitro* geneknockdown assays. Ultimately, our work elucidates a dynamic myeloid cell trajectory post-SCI and identifies a novel subpopulationof *Fabp5*+ inflammatory myeloid cells, comprising both resident and infiltrating cells and displaying a delayed cytotoxic profile at the lesion epicentre, which may serve as a target for future therapeutics.

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T14-008A

Severe experimental autoimmune encephalomyelitis (EAE) is ameliorated by human olfactory-derived mesenchymal stromal cell transplantation revealing a role for IL-16

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Bone marrow-derived mesenchymal stromal cells (BM-MSCs) are being tested in the clinic for the treatment of the demyelinating disease, multiple sclerosis (MS) [1, 2]. Their immunomodulatory action, which targets the

inflammatory component of the disease, is thought to make them ideal candidates. We have identified mesenchymal stromal cells from the human olfactory mucosa termed, OM-MSCs[3]. These cells have additional properties suitable for repair since they also enhance CNS myelination and skew microglia to an anti-inflammatory phenotype *in vitro* [4]. Therefore, OM-MSCs may have therapeutic benefits over BM-MSCs in the treatment of MS. In this investigation, we have compared the reparative properties of OM-MSCs to BM-MSCs *in vivo* using the experimental autoimmune encephalomyelitis (EAE) model. Although, both MSC types were capable of ameliorating disease, if delivered at early disease onset, when administered during established severe disease, only OM-MSCs significantly improved disease outcome. OM-MSCs mediate this action, in part, by a faster closing of the BBB and a reduction in the recruitment of inflammatory cells, which leads to improved remyelination and axonal survival within demyelinated lesions. OM-MSCs also modulated IL-16 secretion of recruited inflammatory cells. Further *in vitro* investigation of IL-16 revealed a novel inhibitory role on OPC differentiation and myelination. Our data suggests that OM-MSCs may have therapeutic benefits over BM-MSCs in the treatment of MS, especially if administered during progressive disease.

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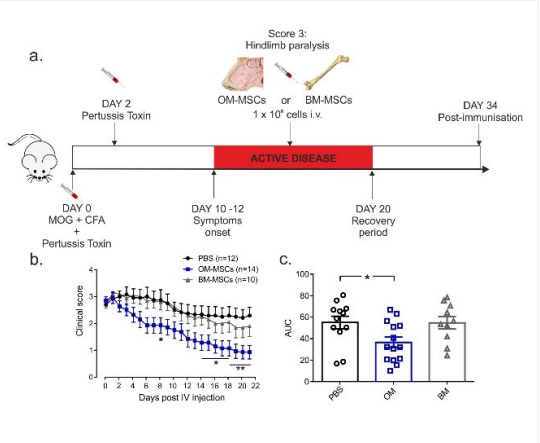

Human OM-MSC and BM-MSC transplantation in severe experimental autoimmune encephalomyelitis (EAE)
a. EAE experimental timeline; injection of human olfactory mucosa (OM)-MSCs or bone marrow (BM)-MSCs (1 x 10^6 cells) or PBS during severe disease (average score 3). b. OM-MSC treatment significantly improved clinical score compared to control animals, BM-MSC treatment had no effect. c. Significant reduction in the area under the curve (AUC) in OM-MSC treated animals compared to control. OM-MSCs produced a positive therapeutic benefit if administered when animals have active demyelination and severe neurological deficits.

T14-009A

Single Cell Sequencing Reveals Glial Specific Responses to Tissue Processing & Post-Mortem Across Species

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The goal of single cell and single nucleus sequencing (scRNA-seq/snRNA-seq) is to analyze the transcriptional profile of cells in their *in situ* state. However, cell dissociation and post mortem processes have the potential to induce artifacts that can mask true cell states or types. While there are no shortage of dissociation protocols in the literature, most have been optimized with a focus on cell yield or viability and have overlooked the effect that dissociation can have on gene expression.

Using scRNA-seq, we demonstrate that enzymatic dissociation of central nervous system (CNS) tissue induces an aberrant gene expression signature predominantly in microglia and other CNS myeloid populations that can confound downstream analysis and mask true *in vivo* microglial cell states. To combat this issue, we have developed a flexible protocol that can be used with fresh tissue samples across digestion protocols, including enzymatic digestion, to eliminate artifactual gene expression.

Additionally, snRNA-seq of human brain tissue revealed that a similar aberrant gene expression signatures are also observed in multiple CNS cell populations from post-mortem tissue. Through integrative analysis of fresh and post-mortem human tissue we identify key aspects of this post-mortem signature in a cell-type specific manner which can be used to guide downstream analyses of published and prospective human datasets. Taken together we provide both a methodological solution, as well as resource dataset for human studies, to better understand and avoid artifacts caused by tissue dissociation or post-mortem tissue.

T14-010A

LRP1-mediated activation of neurotoxic astrocytes and the effect on brain aging

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Changes in gene expression patterns of glial cells can predict brain age[1] suggesting that glial function is a major contributor to changes in cognition which occur due to aging. **Our goal is understand a potential mechanism which underlies age-associated changes in astrocyte physiology.** With aging, astrocytes undergo a series of gene expression changes, switching their neurotrophic role to a neurotoxic role[2]. Neurotoxic reactive astrocytes have disrupted ability to promote synaptic function and improve neuronal survival after stress[3]. Thus, a better understanding of factors which drive the switch into neurotoxic astrocytosis will provide insight into mechanisms which contribute to neurotoxic astrocyte formation during aging, enabling intervention to prevent functional declines in cognition which occur with age.

Neurotoxic reactive astrocytes are induced by microglial secretion of Interleukin-1α (IL1α), Tumor necrosis factor (TNF), and Complement component C1q[3]. Alterations in astrocyte sensitivity to these factors would in turn, increase or reduce the numbers of neurotoxic astrocytes. We have discovered a potential astrocyte mediator, Low-density lipoprotein receptor related protein 1 (**LRP1**). Located on the plasma membrane, LRP1 uses receptor mediated endocytosis to escort a variety of proteins to the endocytic system for degradation, associates with signaling complexes, and is cleaved to regulate nuclear signaling[4]. **Therefore, LRP1 has the potential to fundamentally alter astrocyte response to signals.** We discovered that loss of LRP1 causes a significant increase in astrocyte sensitivity to TNFα. Others found that LRP1 regulates components of the complement system,[5] and interferes with IL1 receptor signaling[6],altogether suggesting LRP1 could directly affect astrocyte sensitivity to microglia signals that activate neurotoxic astrocytes. We discovered that astrocyte-specific knockout of LRP1 in mice causes worse behavioral outcomes when aged to 15 months. We think that this worsened outcome

could be due to neuroinflammation and increased neurotoxic astrocytes. To test this, we are performing protein and gene expression studies in astrocytes lacking LRP1 *in vivo*, while simultaneously interrogating the effect of LRP1 on neurotoxic astrocyte activation *in vitro* and in aged mouse models.

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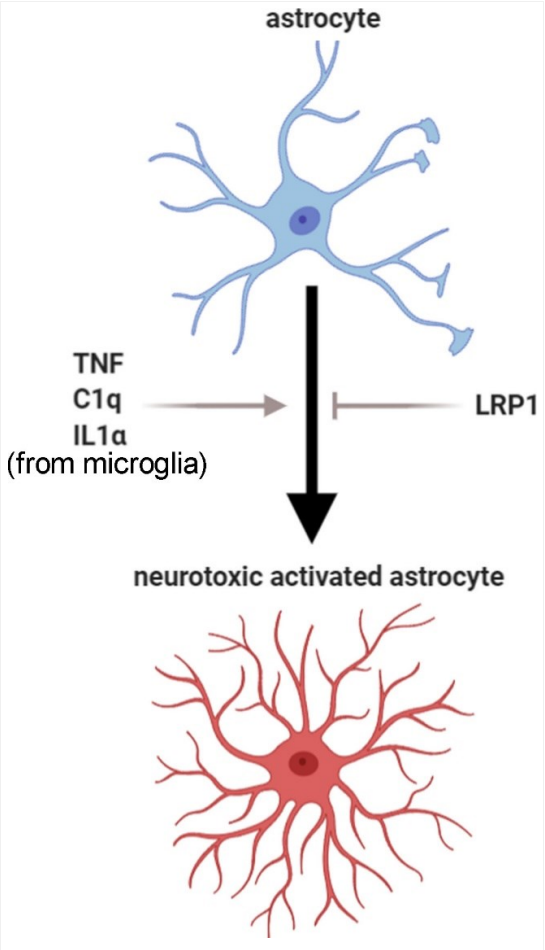

LRP1 as a modulator of astrocyte activation
Fig. 1: LRP1 is proposed to decrease astrocyte sensitivity to cytokines, thereby preventing activation of neurotoxic astrocytes.

T14-011A

Implication of microglial activation in chloride homeostasis impairment, long term consequences of TBI

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Traumatic brain injury (TBI) affects more than 10 million people worldwide each year. The economic impact of TBI is estimated annually, for the United States at more than $221 billion, and more than 33 billion Euros in Europe (Gustavsson et al., 2011), altogether putting TBI as a major public health concern. TBI is a dynamic process, with a temporal course dividing in two phases: An initial phase mainly drives by physical lesions responsible for cellular modifications and then a second phase, delayed in time during which appear memory impairment, together with epilepsy and depression (Nicholl and LaFrance, 2009). It has been proposed that maintenance of intracellular chloride concentrations by specific transporters (NKCC1 and KCC2) plays a major role in the proper functioning of brain inhibition and in the prevention of trauma-induced hyperexcitability, neuronal death and inflammation.

Using a controlled cortical impact (CCI) model on mice, we want to assess long term consequences of TBI on neuroinflammation and cognitive functions. The lesion is located at the level of the parietal cortex of 8-weeks old mice.

We showed impairment in working and episodic memory 1-month post TBI. Treatment of mice using bumetanide, an antagonist of NKCC1, showed a potent action in preventing the occurring of these memory disorders. In the track of recent findings we made (Goubert and al., 2019), we showed that the injury leads to imbalance in the hippocampal secondary neurogenesis in favor of radial glia like cells pool with decrease in neuronal production. Also, we described a massive loss of parvalbumin-containing interneurons already three days after the trauma. We were able to link this interneurons' loss with the secondary neurogenesis changes we observed and with a decrease of microglia cells surveillance. From these observations, we showed that bumetanide treatment influences microglial cell activation inducing polarization towards the proinflammatory phenotype M1, 3 days post-CCI and the anti-inflammatory phenotype M2, 7 days post-CCI.

This study focuses on how chloride homeostasis regulation influences glial cells. For the first time, we show that targeting the chloride co-transporter NKCC1 may act on the activation kinetics of microglial cells. We are thus deciphering an additional pathway to understand the mechanisms of bumetanide action and to understand how long-term sequelae are linked to neuroinflammation after TBI, with the aim of implementing a more complete and effective treatment.

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T14-012A

Glial cell activation and altered metabolic profile in the spinal-trigeminal axis in a model of multiple sclerosis-associated trigeminal pain

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Trigeminal (TG) neuralgia is one of the worst multiple sclerosis (MS)-associated neuropathic pain syndromes, with a 20-fold higher risk in patients than in the general population [1]. TG pain is often an early or even an onset symptom of MS, which does not generally correlate with the severity of the disease and with the presence of demyelinating plaques in central nervous system (CNS) areas involved in pain transmission [2], suggesting that pain and clinical signs in MS are triggered by parallel but independent yet-to-be identified mechanisms. Thus, our aims were to study the development of spontaneous TG pain in an animal model of Experimental Autoimmune Encephalomyelitis (EAE), and to analyze: i) the activation of glial cells (i.e. astrocytes and microglia in the CNS and satellite glial cells in the TG ganglion), and ii) metabolic changes in the spinal-TG system.

EAE was induced in Dark Agouti male rats by intra-dermal injection of recombinant MOG_{1-125} protein fragment in Incomplete Freund's Adjuvant (IFA) and sodium acetate. Motor symptoms were monitored on a 1-7 points scale of ascending paralysis. The development of orofacial allodynia was evaluated by von Frey's hairs. At day post-immunization (DPI) 21 or at the onset of EAE symptoms, animals were sacrificed, and the brainstem, TG ganglia and nerves were collected for analyses of glial cell activation and metabolomics/lipidomics analyses by mass spectrometry.

The subcutaneous injection of recombinant MOG_{1-125} protein fragment to Dark Agouti male rats led to the development of relapsing-remitting EAE, with a first peak after DPI 13, a remission stage from DPI 16 and a second peak from DPI 21. Interestingly, orofacial allodynia developed from DPI 1, i.e. well before the onset of EAE, and worsened over time, irrespective of the disease phase. Activation of glial cells both in the TG ganglia and in the brainstem, together with over-expression of glial purinergic receptors involved in pain transmission, was observed along with metabolic alterations in the TG ganglion, with no signs of demyelination in the brainstem. At EAE onset, brainstem glial cells were already activated and overexpressed the A_3 adenosine receptor subtype [3].Our data show the spontaneous development of TG pain before the onset of relapsing-remitting EAE in rats, suggesting the existence of parallel mechanisms controlling motor symptoms and orofacial pain. The involvement of central and peripheral glial cell activation and metabolic alterations in the TG ganglia suggest their contribution to trigger the sensitization of sensory neurons. Thus, the reduction of glial cell activation and normalization of TG ganglia metabolism are interesting options to manage pain in MS.

Acknowledgement

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T14-013A

Longitudinal MR imaging of locally induced EAE mice revealed the extent of blood-spinal cord barrier disruption as a predictive marker of demyelination severity

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Background: Multiple sclerosis (MS) is an inflammatory and demyelinating autoimmune disorder of the central nervous system (CNS). There are few therapeutic agents to prevent the progression of disability, and for the development of new therapeutic agents, establishing a sensitive biomarker that reflect disease status and therapeutic response is important for conducting precise clinical trial. However, considering the neurological disorders, there are still few fluid diagnostic markers. Magnetic resonance imaging (MRI) is expected to provide information on current status of MS patients including disease activity and disability. Thus, in the present study, we aim to demonstrate the feasibility of MRI as a biomarker for disease activity and functional disability by using mice model of multiple sclerosis.

Method: Mice were immunized with MOG_{35-55} and complete Freund's adjuvant and 3 weeks after that, injected cytokine mixture containing TNF-α and IFN-γ into thoracic spinal cord under anesthesia. Pertussis toxin was administered intravenously 0 and 2 day after the cytokine mixture injection. Dynamic-enhanced contrasted MRI (DCE-MRI) and diffusion tensor imaging (DTI) applied to assess Gd leakage into spinal cord as an index of activity and diffusion indices as an index of disability of spinal cord at 7, 14, 21 days after cytokine mixture injection. The diffusion indices were selected radial diffusivity (RD) as an index of demyelination and axial diffusivity (AD) as an index of axonal damage in white matter.

Results: Based on our data, locally induced EAE mice shows biphasic spinal cord pathology. Gd leakage into spinal cord was observed at acute stage but rarely observed at chronic stage. On the other hand, RD increase in white matter, which indicates demyelination, progress toward chronic stage. The spinal cord pathologies observed in MRI analyses were validated with Evans blue leakage into spinal cord and histological analysis. In acute stage, the neurological symptom correlated with the Gd leakage level, whereas in chronic stage, the symptom correlated with RD increase. We also revealed that Gd leakage level could be used for predictor for RD increase in white matter, which means that blood-spinal cord barrier (BSCB) disruption could be used for prediction marker of demyelination.Conclusion: We revealed that spinal cord pathology of targeted EAE mouse is successfully detected by longitudinal MR imaging, and the extent of BSCB disruption could be used for the predictive marker of demyelination in later phase.

T14-014A

The autophagic protein Beclin1 modulates microglial activation and neuroinflammation

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One of the main features of Alzheimer´s Disease (AD) and other neurodegenerative diseases is a progressive neuroinflammation, mediated by chronic activation of glia cells. In AD, microglia are the main producers of pro-inflammatory cytokines. Autophagy degrades and removes damaged organelles and various proteins, thus presenting a major quality control mechanism of the cell. The loss of the autophagic protein ATG16L1 is associated with Crohn's disease and resulted in enhanced activation of macrophages (Murthy et al., 2014, Nature 506). The autophagic protein Beclin1 is a crucial factor in the nucleation process of autophagosome formation and was shown to be strongly reduced in microglia from AD patients (Lucin et al., 2013, Neuron 70). Based on these data, we assessed the role of Beclin1 in neuroinflammation and AD.
As Beclin1$^{-/-}$ mice die *in utero*, primary microglia were isolated from neonatal Beclin1$^{+/-}$ mice and wild type litter mates. Beclin1$^{+/-}$ microglia released more IL1beta and IL18 than microglia from wild type mice following acute (LPS+ATP) stimulation. Consequently, Beclin1$^{+/-}$ mice crossed to an AD-like mouse model, had also increased IL-1beta levels, but interestingly, no changes in amyloid beta pathology. Subsequent analyis revealed that Beclin1$^{+/-}$ microglia contained more inflammasomes, and elevated levels of NLRP3 and of cleaved Caspase1. Superresolution microscopy revealed a very close association of NLRP3 and the autophagosomal marker LC3-II, indicating that selective autophagy can impact microglial activation by NLRP3 and inflammasome degradation (Houtman et al., 2019, EMBO J 38).
To assess the effects of a complete loss of Beclin1 in microglia *in vivo*, Beclin1$^{flox/flox}$ mice were crossed to Tamoxifen-inducible Cx3cr1^{Cre} mice on a genetic AD-like mouse background. Theoutcome of this microglia-specific Beclin1 deficiency on AD pathology will be presented.

T14-016A

Rifaximin improves neuroinflammation and neurotransmission in cerebellum and hippocampus of rats with mild liver damage

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Hepatic encephalopathy (HE) is a neuropsychiatric syndrome consequence of liver failure. Patients with HE show cognitive and motor impairment and alterations of personality and consciousness. Patients with liver cirrhosis may show minimal hepatic encephalopathy (MHE), with mild cognitive impairment, psychomotor slowing and motor incoordination, which reduce life quality and span. Hyperammonaemia and systemic inflammation produced by the hepatic failure act synergistically to promote neuroinflammation. Neuroinflammation is the main responsible of altered neurotransmission leading to the cognitive and motor impairments in MHE (Cabrera-Pastor et al 2019). Neuroinflammation appears at early stages of liver disease, it was found in cerebellum of patients died with

steatohepatitis, even before reaching cirrhosis (Balzano et al., 2018).

Rifaximin is a non-absorbable antibiotic that improves MHE, restoring cognitive function in many patients and reduces peripheral inflammation. Due to its safety, efficacy and tolerability rifaximin is usually administered to MHE patients.

The aims of this work were to assess neuroinflammation in cerebellum and hippocampus of rats with different grades of liver damage and its effects on neurotransmission and to assess if rifaximin reverses such alterations.

Progressive liver damage was induced by CCl_4 injection during 12 weeks. Rats were treated with rifaximin orally, every day. Rats were sacrificed at 2, 4, 8 and 12 weeks of CCl_4 injections to analyze progression of liver damage (steatosis, inflammation, fibrosis, cirrhosis) and neuroinflammation. Neuroinflammation in cerebellum and hippocampus was assessed by immunohistochemistry analyzing astrocyte and microglial activation and TNFα levels. Neurotransmission was assessed by analyzing membrane expression of glutamate and GABA transporters and extracellular GABA and glutamate in cerebellum.

Rats with liver damage show microglial activation in cerebellum and hippocampus at 2 weeks of CCl_4 administration, with only hepatic steatosis. Microglial activation remains until cirrhosis stage. Rats also show activation of astrocytes starting at 4 weeks, when liver inflammation is also present. Rifaximin reverses glial activation from 3 weeks of treatment. In cerebellum, rats with liver damage show higher levels of TNFα at 4, 8 and 12 weeks and reduced membrane expression of glial glutamate and GABA transporters (GAT1 and GLAST), indicating alterations in neurotransmission. Rifaximin normalizes TNFα levels and membrane expression of glutamate transporters.

These data show that mild liver damage already induces neuroinflammation, which is reversed by rifaximin treatment, suggesting beneficial effects of early treatment with rifaximin in patients with MHE.

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T14-017A

Topographical characterisation of distinct astrocyte populations along the length of the MS neuraxis

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Introduction: Astrocytes are the most abundant and heterogenous glial cell in the central nervous system (CNS). They exert many essential complex functions in healthy CNS and neurodegenerative pathologies. In that context, multiple sclerosis (MS) is a chronic autoimmune and inflammatory demyelinating disease, where demyelination occurs in both the cerebral cortex and spinal cord. It is marked by glial cell activation-dysfunction leading to substantial and irreversible disability. Moreover, carriage of *HLA-DRB1*15,* the primary genetic risk factor for MS, has been shown to increase neuroinflammation and demyelination. Despite their crucial role in immune regulation and neurotrophic support, astrocytes have been underestimated as early and active contributors of MS pathology. Astroglial subtype diversity throughout the CNS ideally positions them to be involved across the different levels of the neuraxis and may shed light on MS heterogeneity. Thus, this study is focused on astrocyte topography along the MS neuraxis and its relationships with genotype in secondary progressive MS patients cohort.
Method: A cohort of pathological confirmed MS (n=20; *HLA-DRB1*15+, n*=10; *HLA-DRB1*15-*, *n*=10), and non-neurological control cases (*n*=10) was used. Adjacent formalin-fixed, paraffin-embedded motor cortical, cervical, and lumbar spinal cord sections were immunolabelled using specific astrocytes markers (ALDH1L1, GFAP). The astrocyte density throughout the cohort and sub-groups are reflected by pixel densities (pixel/mm^2) which is used to assess astrocyte expression in pre-defined fields of view spaced at systematic intervals of the motor cortex and spinal cord levels. Comparisons between grey and white matter as well as age dependence was conducted.
Results: MS motor cortical and spinal cord ALDH1L1 expression was greatest in the grey matter (p<0.0001), whereas GFAP expression was greatest in the white matter (p<0.0001)**.** Astrocytes labelled with GFAP marker were influenced by *HLA-DRB1*15* status, especially in cases equal to or younger than the median age of 62-years old, with *15+* individuals expressing greater GFAP expression than their *15-* counterparts (p=0.014)**.** Interestingly, astrocyte expression modified in lesional and perilesional areas was restricted to GFAP-positive astrocytes.**Conclusion:** The striking differences between ALDH1L1 and GFAP-positive astrocytes in MS show distinct astrocyte populations. Based on the consistent astroglial expression across the neuraxis, we suggest that astrocyte dysfunction, namely GFAP-positive astrocytes, diffusely contribute to worse MS pathology across the CNS rather than targeting specific regions. These different roles astrocyte subtypes play in MS pathology will be crucial to understanding MS heterogeneity.

T14-018A

Tmem119 is a useful marker for studying human microglia heterogeneity in age-related brain pathology

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Microglia, the resident immune cells of the central nervous system (CNS), play multiple roles in CNS homeostasis during development, adulthood, and aging under both physiological and pathological conditions. Microglia are traditionally thought to be a heterogeneous population, which allows these cells to achieve a wide range of responses to ever-changing environments. However, the relevance of microglial morphological changes to human brain health and disease is still unclear. Here, we analyzed microglia morphologies in the human cerebral cortex during normal aging versus Alzheimer's disease (AD) related pathology. The tissue samples were obtained from autopsied brains of men and women aged 70 to 98 years (n=8). The antibodies against transmembrane protein 119 (Tmem119), a newly identified high-specific marker protein of microglia, were used to label microglial cells.
By light microscopy and confocal laser scanning microscopy, we have demonstrated that microglial cells immunostained for Tmem119 are morphologically heterogeneous in both AD and non-AD cases. In non-AD aged

brain samples, ramified microglial cells with numerous thin and branching processes are predominant. Some cortex microgliocytes show signs of activation, such as body enlargement and processes thickening. The presence of long rod-shaped microglial cells is also noted. In AD samples, most microglial cells outside the amyloid plaques are also characterized by ramified morphology. Microglia, which form a clusters around amyloid plaques, have enlarged somas and reduced ramification. Microglia processes are frequently found wrapping around the amyloid plaque and penetrating into it. Plaque-associated microglia also express Tmem119 (fig.1). Interestingly, Tmem119 appeared to be discretely distributed in the body and processes of microglial cells in all cases. Using super-resolution confocal microscopy, we found that Tmem119 forms discrete microclusters (less than 1 μm in size) in the plasma membrane of microgliocytes.

Further understanding mechanisms and functional meaning of microglia heterogeneity may offer therapeutic opportunities for a broad spectrum of CNS disorders. Tmem119 seems to be a useful marker for research in this field.

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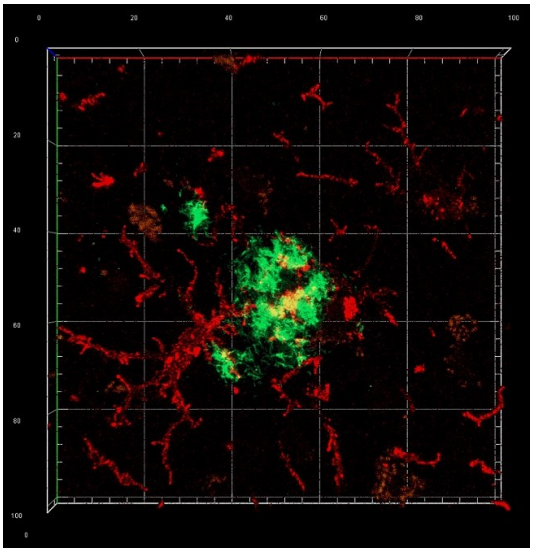

Figure 1. Tmem119 and amyloid beta double-labeling of AD human cerebral cortex. Microglial cell with enlarged soma and reduced ramification (red) extends the processes to amyloid plaque (green).

T14-019A

Plasmalemmal localization of MHC class II molecules in interferon γ treated astrocytes is mediated by reversible exocytosis of larger vesicles and prolonged by inhibited endocytosis

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AIM: Major histocompatibility complex class II molecules (MHCII) are pivotal in immune responses as they present antigens to T cells. Interferon γ (IFNγ), an inflammatory cytokine, induces MHCII expression in several cell types including astrocytes. We here examined vesicular mechanisms involved in MHCII delivery to the plasmalemma and their retention on the surface of IFNγ-treated astrocytes.
METHODS: Cultured neonatal rat astrocytes were treated with 600 U/ml of IFNγ for 48h. Confocal microscopy was used to evaluate the quantity and plasmalemmal localization of immunolabeled MHCII. High-resolution, cell-attached patch-clamp capacitance measurements were used to examine elementary events of exo-/endocytosis.
RESULTS: IFNγ treatment increased MHCII expression in astrocytes; the relative proportion of MHCII-immunopositive cell area increased by ~eightfold (from 1.5% to 11.6%; P<0.001). The plasmalemmal localization of MHCII was detected only in live, non-permeabilized astrocytes treated with IFNγ. In these astrocytes, larger vesicles underwent reversible exocytosis, whereas the frequency of full endocytosis was reduced. Stimulation with 100 μM ATP that increases the free intracellular Ca^{2+} concentration modulated elementary exo-/endocytotic activity; the frequencies of reversible and full exocytosis increased, whereas the frequency of full endocytosis decreased. In IFNγ-treated astrocytes ATP-evoked alterations of exo-/endocytotic activity were largely conserved.CONCLUSIONS: IFNγ induces expression of MHCII that translocate to astrocyte surface via larger vesicles preferentially entering reversible exocytosis. Concomitant inhibition of full endocytosis seemingly prolongs MHCII exposure at the astrocyte surface. The modulation of elementary exo-/endocytosis by ATP is largely intact in IFNγ-treated astrocytes.

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T14-020A

Searching for key factors behind the severity of multiple sclerosis: new advances in the functional characterization of the myeloid-derived suppressor cells.

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells with a regulatory role in immune-related disorders, including multiple sclerosis (MS). In the context of the experimental autoimmune encephalomyelitis (EAE), MDSCs are involved in T cell suppression within the CNS. Our group recently published that the proportion of monocityc MDSCs is related to the severity of the EAE clinical course and tissue damage

extent. For the first time, our group has demonstrated the presence of MDSCs in the CNS of MS patients, being mainly circumscribed to areas with a spontaneous capacity of remyelination. Interestingly, our preclinical data pointed to MDSCs as bioindicators of the future clinical course severity since the abundance of MDSCs in the peripheral blood at the onset of the symptoms is inversely correlated with the severity of the clinical course, as well as with demyelination and axonal damage.In the present work, we perform in vivo and vitro approaches using both human MS samples and the EAE model with the goal of investigating if the functional characterization of MDSCs might also help us to shed more light on the variability in the clinical course of the disease. In this sense, the correlation between MDSCs and the viability of T cell was analyzed in CNS from 11 MS patients with different aggressiveness of their clinical courses. Interestingly, preliminary data showed a direct correlation between the abundance of MDSCs and apoptotic T cells in active lesions. Furthermore, the higher density of non-viable T cells seemed to be related to a milder clinical course according to the disease length. Thus, we considered studying functional differences of MDSCs on the EAE clinical course severity. We firstly performed clustering analysis of our clinical and immunological data at onset of the symptoms in the EAE model, identifying two groups of mice with different clinical course severity. We corroborated that differences in the abundance of MDSCs in both groups of EAE mice were not only numerical, but also functional, i.e. different degree of immunosuppression activity in vitro. In order to go in depth in the functional differences of MDSCs, we have preliminary transcriptomic data indicating that MDSCs isolated from EAE mice with a severe clinical course seemed to have a differential transcriptomic profile. In sum, our results seem to indicate that the clinical course severity would be related not only to the abundance of MDSCs but also to a different immunosuppressive function of this cell population, likely due to a different genomic profile. In this sense, our data suggest that MDSCs with better immunosuppressive functions might be used as a new cell-based therapy to efficiently modulate the aggressiveness of MS.

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T14-021B

Cortistatin: a key factor involved in the molecular and cellular mechanisms of the healthy aging

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Aging is a multifactorial, progressive and unavoidable process that affects all societies. In 2050 more than 2000 million people will have more than 60 years of age. Between the main consequences of aging are the alterations in the immune system (IS) and central nervous system (CNS). While IS may interfere with brain function, CNS can also modulate immune responses, and both can sense internal and external changes and communicate in a bidirectional way. Therefore, the search for endogenous factors that can regulate the cross-talk between IS and CNS to evaluate immune and neural interactions and dysfunctions throughout aging is crucial. Accordingly, we

decided to study the endogenous role of cortistatin (CST), an immunomodulatory/neuroprotective neuropeptide expressed by both glial cells and peripheral immune system, in the cellular/molecular changes associated to aging. Previous studies from our group have shown that CST deficiency generates a proinflammatory profile in the peripheral immune responses and induces higher susceptibility in preclinical models for some neurodegenerative conditions (such as Parkinson and demyelinating multiple sclerosis). We also found decreased levels of CST in peripheral samples from neurodegenerative diseases such as Alzheimer´s and Huntington´s, and in diseases with premature aging of the CNS (Cushing, epilepsy). Here, we investigate the role of CST in peripheral and central immune activation in aged animals. We have observed a higher reduction in the number of cortical neurons, increased microgliosis and astrogliosis, and greater reduction of oligodendocytes in the corpus callosum of CST-deficient mice than in WT naïve mice. Moreover, morphometric analysis reveals that CST deficiency results in premature activated amoeboid microglia morphology with short processes and big somas when compared with aged-matched WT mice. Accordingly to this, when CST is missing, molecular analysis of mice brains showed an increased gene expression of pro-inflammatory mediators but a decrease in the expression of trophic factors. In addition, by using primary adult glial cells isolated from CST-deficient mice, we observed that lack of CST induced an activated phenotype with deregulated responses to inflammation (after incubation with bacterial endotoxin), hypoxia and hypoglycemia. In summary, we identify CST as a key factor in the neuro-endocrine-immune axis able to regulate tissue-surveying function of glial cells in a healthy brain, whose absence generates a phenotype of premature and/or exacerbated aging, especially affecting neuroinflammatory responses.

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T14-022B

TAM receptor signalling inhibits induction of GM-CSF in microglia undergoing pro-inflammatory stimulation

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Neuroinflammation is implicated in many disorders such as multiple sclerosis and Alzheimer's disease. Microglia, the resident immune cells of the CNS, have an important role in regulating inflammation so that permanent damage is not caused to the brain. The TAM (Tyro3, Axl, Mer) receptor tyrosine kinases are believed to be regulators of the CNS microglial response to inflammation and immune infiltration; however, the exact mechanisms of their actions remain to be fully determined. Here, we aimed to investigate the role of TAM signalling in modulating the CNS glial expression of the haematopoietic growth factor and immune modulator, GM-CSF, under pro-inflammatory conditions.

Primary cell cultures were set up using neonatal C57/BL6 wild-type mouse brains from which microglia were derived through selective plating. All animal procedures were approved under a UK Home Office project licence. A gene array targeting 84 different genes from the Toll-like receptor-signalling pathway was used to identify any genes that changed substantially when cells were primed with 1.6μg/mL Gas6 (TAM ligand) for 1 hour before a further 8-hour incubation with 10ng/mL of the pro-inflammatory substance, lipopolysaccharide (LPS). *Csf2*, encoding GM-CSF, was identified as a key gene that was altered by Gas6 treatment.

Further RT-qPCR experiments were used to explore the profile of *Csf2* expression over the course of inflammatory stimulation of microglia. GM-CSF protein release into conditioned media was also measured over the same times by ELISA, with Gas6 being tested for its ability to suppress protein release. Interestingly, there was a substantial difference between the peaks of gene expression and protein release after LPS-induced stimulation, which were 8 hours and 48 hours, respectively. Furthermore, Gas6 caused *Csf2* gene expression to reduce to almost half the LPS-stimulated levels, replicating what was initially shown in the gene array. It was also expected that this suppression would be replicated in protein release after 48 hours of LPS-induced stimulation.Together, these data suggest an important role for GM-CSF in the microglial response to inflammation. Gas6, through the TAM receptors, shows a promising potential to modulate this pro-inflammatory response. Future work will investigate the time-dependent changes in gene expression and protein release of GM-CSF, exploring the mechanisms involved. In this way, fully elucidating the relationship between Gas6/TAM signalling and GM-CSF function in the CNS could present a novel route for treatment of neurological disorders that feature neuroinflammation.

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T14-023B

Oligodendrocytes regulate the adhesion molecule ICAM-1 in neuroinflammation

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Neuroinflammation is a complex response to external stimuli with the physiological intent to foster tissue repair after an insult. However, it may become chronic and detrimental, rendering it a hallmark for many neurological diseases. Normally, neuroinflammation is orchestrated by central nervous system (CNS) resident cells. Yet, peripheral auto-reactive lymphocytes may additionally breach into the CNS thus contributing to this process. In multiple sclerosis (MS), autoimmune infiltrating lymphocytes attack the myelin layer as well as the myelin producing cells, known as oligodendrocytes (OI), culminating in demyelinating lesions. Chronic demyelination may result in remyelination failure and consequently in progressive neurodegeneration. Recently, OI have been attributed potential immunomodulatory effects. Yet, the exact mode of interaction with pathogenic infiltrating lymphocytes remains unclear to date. Here, we delve into understanding mechanisms of OI modulation during neuroinflammation and characterize the interaction of OI with pathogenic T cells. We performed RNA sequencing on OI isolated from the model disease experimental autoimmune encephalomyelitis (EAE). These OI significantly upregulated 398 genes in comparison to control OI, many of them belonging to categories related to immune modulation. Conventional qPCR analysis confirmed these results and revealed an upregulation of adhesion molecules ICAM-1 and VCAM-1 in OI from the EAE model. Regulation of adhesion molecules was not observed in the toxic demyelinating cuprizone model suggesting a role of the peripheral immune cell infiltration in the upregulation of adhesion molecules. To explore whether adhesion molecules are involved in the interaction of OI with infiltrating T cells, we

performed co-culture studies on mature OI and Th1 cells. Here, we observed a direct interaction between both cell types: 80% of Th1 cells contacting OI created contacts lasting longer than 15 min, which may be regarded as physiologically relevant. Exposure of OI to Th1 cells or their supernatant resulted in a significant extension of OI processes, and upregulation of adhesion molecules ICAM-1 and VCAM-1 as well as other immunomodulatory genes. Consequently, blocking of oligodendroglial ICAM-1 reduced the number of Th1 cells initially contacting OI, but did not affect the creation of stable contacts. These results suggest that adhesion molecules may play a role in the interaction between OI and T cells. Further characterization and understanding of OI interaction with infiltrating cells may lead to new therapeutic strategies enhancing OI protection and remyelination potential.

T14-024B

Ageing as a key regulator of the interaction between the immune system and oligodendrocyte progenitor cells

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During CNS remyelination, adult oligodendrocyte progenitor cells (OPCs) are recruited to the demyelinated area and differentiate into new myelinating oligodendrocytes that ensheath axons. Like all regenerative processes in mammals, remyelination efficiency declines with ageing leading to neuronal loss and accrual of permanent disability.

Recent publications have highlighted the wide heterogeneity of oligodendrocyte lineage cells in demyelinating models as well as MS patient samples, including a previously unrecognised immune-like oligodendrocyte lineage cluster. Here we show that neonatal OPCs can reduce the expression of inflammatory cytokines, such as IFN-g, by activated $CD4^+$ T cells without affecting expression of the anti-inflammatory cytokine, IL-10. We also show that this immune modulation is soluble, as neonatal OPCs reduce IFN-g expression by $CD4^+$ T cells. Decreased IFN-g expression is independent of MHCII expression by OPCs, suggesting a novel OPC-CD4 T cell interaction mechanism. We also show that this OPC-mediated immune-modulatory process is impaired with ageing, even though aged OPCs have high levels of expression of proteins associated with immune processes.

These results reveal an OPC-mediated immune modulatory mechanism contributing to the regulation of T cell-mediated inflammation. The age-associated impairment of OPC immune-modulation may contribute to OPC insufficiency and remyelination failure leading to MS disease progression. Exploring this mechanism will provide new insights into MS pathogenesis as well as new therapeutic targets.

T14-025B

Differential Functional Contributions of Microglia and non-parenchymal CNS macrophages in physiology and pathophysiology

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Brain macrophages have emerged as major players in central nerve system (CNS) physiology and pathophysiology. Much of the recent insight derives from fate mapping, intra-vital imaging, cell ablation and targeted mutagenesis using respective Cre / loxP system-based mouse models. In parallel, advances in flow cytometry and single cell transcriptomics have highlighted the complexity of the brain macrophage compartment. Specifically, the latter comprises parenchymal microglia and non-parenchymal, CNS border-associated macrophages (BAM) located in perivascular and meningeal niches, as well as the choroid plexus. In order to understand specific functional contributions of these distinct CNS macrophage populations we applied a novel binary transgenic split Cre approaches that allows to dissect parenchymal microglia and meningeal perivascular macrophages (mPVM) in physiological and pathophysiological context (*Kim et al., 2021*).

Here we report on our ongoing efforts to uncover the specific role of BAM in steady state and under CNS pathophysiology. Translatome profiling using the 'Ribo-tag' allele (*Haimon et al., 2018*), reveals that mPVM express factors involved in extra cellular matrix remodeling, including substrates, enzymes and modulators. To probe for the functional role of mPVM in controlling blood-brain barrier (BBB) integrity and CNS entry of leukocytes during neuroinflammation, we use the experimental autoimmune encephalomyelitis (EAE) paradigm, a mouse disease model of multiple sclerosis.

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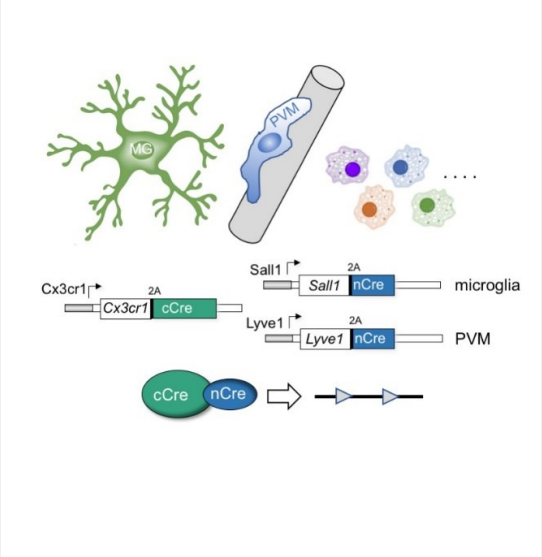

Tackling tissue macrophage heterogeneity
The CNS harbors multiple distinct macrophage populations, including parenchymal microglia and non-parenchymal cells, individual functions of which remain imcompletely understood.

We recently established a binary transgenic mouse model that allows differenctial targeting of these cells and their study in CNS physiology and pathophysiology.

T14-026B

Analysis of Neuroinflammation in CCL17-DTR Mice following DT-treatment

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The chemokine CCL17 is best known for its role in facilitating interactions between T cells and dendritic cells. Previous research in our lab demonstrated homeostatic expression of CCL17 in murine hippocampal neurons. Analysis of the tissue-resident macrophages of the brain, the microglia, showed that CCL17 is required to maintain the homeostatic morphology of these cells. A deficiency for CCL17 resulted in an activated phenotype.

Ablation of CCL17-expressing cells by i.p. injection of Diphtheria toxin (DT) in mice expressing the DT receptor (DTR) under the control of the *Ccl17* promotor (CCL17-DTR) results in the development of epileptic seizures. Seizures emerged and peaked around day 7 post-DT and sustained with reduced frequency for at least 28 days post-DT (Fig 1). The hippocampi of CCL17-DTR mice undergoing seizures are characterized by a loss of hippocampal neurons as well as inflammatory responses of brain resident cell populations, particularly microglia and astrocytes. In addition, strong fluctuations in body weight were observed in these mice.

Onset and development of neuroinflammation following the DT-mediated ablation of $CCL17^{+}$ neurons in CCL17-DTR mice was monitored by immunohistology. Neuronal cell loss was analyzed by H&E and Fluoro Jade C staining. DT was administered one or three times to compare the efficiency of the ablation. The integrity of the blood-brain-barrier (BBB) was analyzed by Evans Blue dye extravasation.

Ablation of $CCL17^{+}$ cells induced extensive neuronal cell loss in the hippocampus as well as micro- and astrogliosis in the first two weeks post-DT (Fig. 2). One time DT-administration was sufficient to induce neuroinflammation or neuronal degeneration. Shortly after DT-injection, the BBB remained intact in CCL17-DTR mice following DT-treatment.

In conclusion, this model is less invasive than common epilepsy mouse models, e.g. the kainic acid model and could, in accordance with the 3R principle, reduce the harm inflicted on animals. Ablation of CCL17-expressing neurons in CCL17-DTR mice serves therefore as a promising model for the analysis of inducible neuroinflammation associated with TLE.

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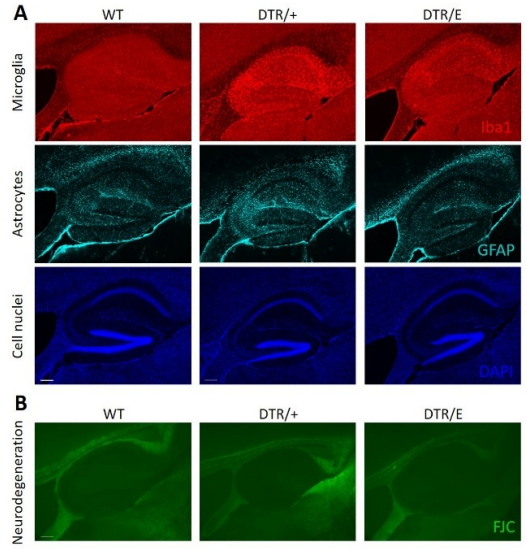
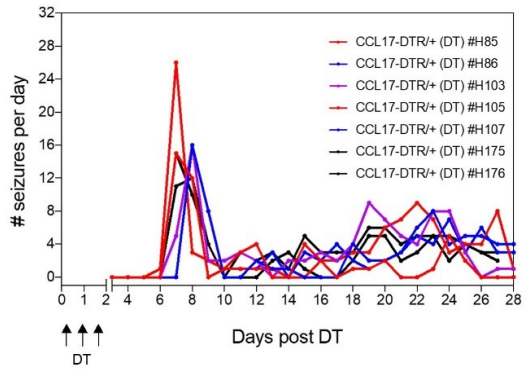


Microgliosis precedes astrocyte activation in early neuroinflammation in CCL17-DTR/+ mice
WT mice, CCL17$^{DTR/+}$ mice and CCL17$^{DTR/E}$ mice received 0.4µg DT i.p. at days 0, 1, and 2. Mice were perfused in situ and brains were isolated at day 5. 40µm brain sections were prepared and stained for the microglia marker IBA-1, astrocyte marker GFAP and counterstained with DAPI (**A**), or stained for degenerating neurons by Fluoro Jade C (**B**). Images were prepared using epifluorescence microscopy. Scale bar (250µm) applies to all panels. WT = wild type mice, DTR/+ = CCL17$^{DTR/+}$ mice, DTR/E = CCL17$^{DTR/E}$ mice; FJC = Fluoro Jade C staining. Representative images are shown (n=3).



Reproducible development of epileptic seizure frequency in CCL17-DTR/+ mice following DT-treatment
Female CCL17$^{DTR/+}$mice received 0.4µg DT i.p. at day 0, 1, and 2. At day 2, mice were anesthetized and a telemetric transmitter was placed into a subcutaneous pocket in the right abdominal wall. Skull surface electrodes were implanted to record electrographic seizures. Individual mice were placed on radio receiving plates, which captured signals from the electrodes and sent them to an input exchange matrix. The digital output of the receiver was converted in real-time into a calibrated analog output. Depicted are the results from three independent experiments (n = 7).

T14-027B

Protective role of T cells in a mouse model of temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is a chronic neurological disease affecting brain structures implicated in cognitive functions. The disease is manifested by the appearance of disabling, spontaneous, recurrent seizures caused by the hyper-excitability and hyper-synchronization of a more or less extensive group of neurons in the temporal lobe structures. Seizures are accompanied by neuronal death, gliosis and structural and synaptic reorganization. Sixty percent of patients do not respond to anti-epileptic drugs and the only curative intervention is the surgical resection of the epileptic focus. It is therefore urgent to better understand the mechanisms involved in pathogenesis in order to identify new therapeutic targets.

It is now well documented that neuroinflammation is critically involved in this disease. Accumulated data suggest that microglia develops a dual profile, pro and anti-inflammatory, and that the balance between these two states

determines the evolution of the disease. Using a TLE mouse model lacking T cells (CD3 -/- mouse), we observed a loss of the microglial anti-inflammatory response and an increase of neuronal death in the hippocampus together with an increase of spontaneous seizure frequency. Our working hypothesis is that, interactions between microglia and T cells drive a protective anti-inflammatory effect in the epileptic hippocampus. Regulatory T cells (Treg) have been shown to promote anti-inflammatory reactions in different models of CNS disorders. In preliminary experiments we therefore depleted Treg by administrating anti-CD25 antibodies during early epileptogenesis and we observed a loss of the microglial anti-inflammatory response and an increased neuronal death. We will confirm these results and test whether this treatment alters the development of seizure events. Eventually, we will assess whether microglial depletion phenocopies T cells depletion worsening TLE. These findings may allow the identification of new therapeutic targets to enhance the endogenous anti-inflammatory response and ultimately improve the treatment of ELT.

T14-028B

The role of enteric glia cells in acute gut inflammation

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Background

Enteric glial cells (EGCs) are neurosupportive cells in the enteric nervous system (ENS) and play an important role in the maintenance of gastrointestinal (GI) physiology. Recently, EGCs have been shown to also exert immune- pathophysiological roles in acute and chronic intestinal inflammation by developing a gliosis-like status. This enteric gliosis is induced by different inflammatory stimuli and shapes the inflammatory environment in the GI tract. The aim of this study is a detailed characterization of EGC- specific response during inflammation *in vivo* by applying the *RiboTag* method, a transgenic model that enables mRNA precipitation from target cell types via the Cre/loxP system.

Methods

We utilized Rpl22-HA (*RiboTag*) mice crossbred with Sox10^{CreERT} mice to generate animals expressing HA- tagged ribosomes in EGCs. Those mice enable the purification of mRNA from EGCs by Immunoprecipitation from the small bowel. Subsequently, we characterized EGC activation in an animal model for acute gut inflammation, the post- operative ileus model (POI), by immunohistochemistry, Western Blot and *RiboTag*-dependent gene expression analysis.

Results

During inflammation, EGCs show a distinct activation pattern with an increased proliferation rate and a gradual increase of gliosis associated genes on protein-level. Our RNA sequencing data of EGC-specific *RiboTag* mRNA confirms an inflammation induced enteric gliosis and can describe for the first time precise glial transcriptional changes in the process of acute gut inflammation. Throughout defined disease stages, EGC transcripts exhibit both an increased response to and an elevated production of certain inflammatory mediators, like cytokines and chemokines. Moreover, EGCs show a clear involvement in occurring migratory processes during POI by expressing several cell motility regulating genes. These and other glial changes are in concordance with inflammation-induced motility impairments and the infiltration of Myeloperoxidase^{+} leukocytes in the small intestine, both hallmarks of POI.

Conclusion

By applying a glia-specific analysis, we were able to describe and interpret EGC responses during acute intestinal inflammation by generating the first reliable gene profiling for an enteric gliosis. Furthermore, our work combines this unique gliosis profile with defined disease hallmarks to elucidate the role of EGCs in different disease stages. Our findings support many recent studies referring to a “reactive” enteric glia phenotype in an inflamed gastrointestinal tract and conceive a list of so far unknown glia-specific genes. Our results can be a starting point for developing new targets to counter gliosis and associated signaling cascades to prevent or mitigate common symptoms in inflammatory gut diseases.

Acknowledgement

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T14-029B

Zika virus infection triggers differential responses in human brain cells

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In the peak of the latest Zika virus outbreak, the cases of the fetal CNS malformations in humans, known as microcephaly, were unambiguously linked to Zika virus (ZIKV) infection via vertical transmission from mother. ZIKV belongs to the family *Flaviviridae*, which comprises of many dangerous human pathogens that may cause severe neuroinfections. The knowledge about flavivirus infections of the CNS tissue, and about the consequences of infection to the morphological and physiological responses of neurons and astrocytes alike, is still fragmental.

Astrocytes are due to their anatomical position among the first cells to intercept the flavivirus after it breaches into the CNS by traversing endothelial cells. In this study, we tested the hypothesis that astrocytes are the key target for ZIKV infection. We compared the selected responses of primary astrocytes, neurons and several cell lines after ZIKV infection. Human fetal astrocytes and neurons from mid-gestation period, the stage of extensive neurogenesis and gliogenesis in the cerebral cortex, were first infected with different strains of ZIKV. Then, we measured the extent of viral infection, release of productive virus, cell survival, and intracellular traffic of ZIKV-laden endocytotic vesicles.

Our results show that human fetal astrocytes are more susceptible to ZIKV infection than neurons (higher infection

rate), they release more progeny virus, but tolerate higher virus loads than neurons. In contrast, in neurons, virus production and release appear to be suppressed with prolonged infection with ZIKV. In addition, we have observed ZIKV strain-dependent responses of the CNS cells. The infection of astrocytes and neurons by ZIKV strains Brazil 2016 (ZIKV-BR), French Polynesia 2013 (ZIKV-FP), and Uganda #976 1947 (ZIKV-UG), has revealed differences in the infection rate and the trafficking of endocytotic vesicles. ZIKV-UG strain was the most infective in neurons and astrocytes, closely followed by ZIKV-BR strain that displayed similarly high infectivity in astrocytes. Both of these strains also elicited faster trafficking of ZIKV-laden endocytotic vesicles in astrocytes and neurons.
Taken together, our results show that astrocytes are the major producer of ZIKV progeny among the brain cells, and that the spread of ZIKV in the brain is affected by the type of the virus strain, which is intimately linked to diverse patterns of the virus dynamics inside and outside the cell.

T14-030B

Direct and indirect roles of different AQP4 isoforms in rapid volume changes in astrocytes

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Water channel aquaporin 4 (AQP4) plays a key role in the regulation of water homeostasis in the central nervous system (CNS). Here, AQP4 is predominantly expressed in astrocytes lining blood–brain and blood–liquor boundaries. While the exact role and location of the isoforms M1 (AQP4a) and M23 (AQP4c) in astrocytes are well established, very little is known of the other AQP4 isoforms.
By using super-resolution structured illumination microscopy, we have shown that, similar to AQP4a and AQP4c, also AQP4e is located in the plasmalemmal supramolecular structures, termed orthogonal arrays of particles (OAPs). On the other hand, AQP4b and AQP4d exhibit extensive cytoplasmic localization in early and late endosomes/lysosomes and in the Golgi apparatus, while neither of the two isoforms constitute OAPs. Nevertheless, all three AQP4 isoforms (AQP4b, AQP4d and AQP4e) affect the density of OAPs. In contrast to the overexpression of AQP4e, which increased the density of OAPs, the overexpression of QP4b or AQP4d reduced the density of OAPs. Brain edema mimicking hypoosmotic conditions triggered a translocation of AQP4e to OAPs and of AQP4d to early endosomes. The observed redistribution of AQP4e and AQPd, following hypoosmotic stress, timely coincided with increased trafficking of vesicles containing respective AQP4 isoforms. In part, the observed changes may be attributed to the alterations in the cytoskeleton, as we have observed rearranged actin filaments in the model of reactive astrocytes and vimentin meshwork depolymerization in hypoosmotic conditions. Finally, our results revealed that AQP4e affects the mechanism of regulatory volume decrease (RVD), while AQP4d facilitates the kinetics of cell swelling, without affecting the RVD in hypoosmotic conditions in astrocytes.Our results suggest that, in addition to isoforms M1 (AQP4a) and M23 (AQP4c), also isoforms AQP4b, AQP4d and AQP4e play an important role in astrocyte water homeostasis (Lisjak et al., 2017; Lisjak et al., 2020). AQP4e incorporates directly into OAPs and thus has an active role in the regulation of OAP structural dynamics and in water homeostasis. On the other hand, AQP4b and AQP4d, which are located exclusively intracellularly, play an indirect role in volume changes in astrocytes. It appears that early endosomes, which lay at “the crossroad” of sorting of different AQP4 isoforms that travel to/from the plasmalemma, are critical for regulating the abundance of OAPs in astrocytes.

Acknowledgement

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T14-031B

BTK signaling regulates microglia-oligodendrocyte interactions during immune-mediated cortical demyelination

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Multiple Sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) characterized by inflammatory demyelination and axonal injury. Cortical demyelinating lesions contain activated microglia and phagocytic macrophages, yet real-time understanding of the role of these cells in driving or responding to demyelination remains elusive. A prominent modulator of microglia function, Bruton's Tyrosine Kinase (BTK), is an active pharmaceutical target for MS treatment; however, how BTK inhibition alters microglia-oligodendrocyte interactions remains unclear. To answer these questions, we developed an inducible *in vivo* model of focal cortical demyelination by applying MS patient derived recombinant antibodies (rAbs) and human complement (MS rAb-huC) to the cortex of mice. Using a combination of *in vivo* two-photon microscopy and single-cell RNA sequencing (scRNA seq), we characterized the dynamics of microglia-oligodendrocyte interactions throughout immune-mediated demyelination and the effects of BTK-inhibition on these interactions and their underlying genetic pathways. MS rAb-huC application induced myelin and oligodendrocyte loss within 72 hours, followed by remyelination over the subsequent 21 days. Demyelination depended on epitope specificity, complement, and an intact Fc receptor binding region in the MS recombinant antibody, suggesting a role for local phagocytic cells to complete demyelination. Indeed, *in vivo* imaging of triple-transgenic mice that allow concurrent visualization of microglia and oligodendrocytes (*Cx3Cr1-CreER;Ai9;MOBP-EGFP*) revealed that microglia rapidly engulfed myelin at the site of antibody application. Oral administration of a brain-penetrant BTK-inhibitor vastly reduced both myelin engulfment and microglial density changes. scRNA sequencing of microglia from MS rAb-huC-affected cortices revealed upregulation of disease-associated pathways that were markedly attenuated with BTK inhibition. In addition, BTK-inhibition broadly reduced inflammatory and increased homeostatic microglia populations. Finally, BTK-inhibition was associated with a large reduction in myelin loss that was indistinguishable from age-matched mice treated with control rAb-HuC. Taken together, our findings point toward a critical role for microglia in not only responding to but driving immune-mediated demyelination.

T14-033B

The protective effect of Angiotensin AT2-receptor stimulation in Neuromyelitis optica spectrum disorder is independent of astrocyte-derived BDNF

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Neuromyelitis optica spectrum disorder (NMOSD) is an antibody-mediated autoimmune inflammatory disease of the central nervous system (CNS), resulting in primary astrocytopathy. We have reported that stimulation of the Angiotensin AT2-receptor (AT2R) with the specific agonist Compound 21 (C21) attenuated NMOSD-like pathology. Recent studies have proposed that the mechanism behind protective effects of AT2R involves brain derived neurotrophic factor (BDNF). Astrocytes are a major cellular source of BDNF. In this study we used mice with conditional BDNF deficiency in astrocytes (GfapF) to examine the involvement of astrocyte-derived BDNF in NMOSD-like pathology and in mediating the protective effect of AT2R stimulation. Anti-aquaporin-4 IgG (AQP4-IgG) from an NMOSD patient and human complement (C) were co-injected intrastriatally to GfapF and wildtype littermate $BDNF^{fl/fl}$ mice (WT), together with either C21 or vehicle at day 0, followed by intrathecal injection of C21 or vehicle at day 2 and tissue collection at day 4. Intracerebral/intrathecal injection of C21, alone or in combination with AQP4-IgG + C, induced BDNF expression in WT mice. Injection of AQP4-IgG + C induced NMOSD-like pathology, including loss of AQP4 and GFAP. There was no difference in the severity of pathological changes between GfapF and WT mice. C21 treatment significantly and equivalently ameliorated NMOSD-like pathology in both WT and GfapF mice. Our findings indicate that astrocyte-derived BDNF neither reduces the severity of NMOSD-like pathology nor is it necessary for the protective effect of AT2R stimulation.

T14-034B

Hops-based Extract with Anti-oxidant Properties Shapes Neuroinflammation in Aged CNS

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Recently, ageing has been associated with an increased systemic inflammation. It has been shown that a chronic inflammatory environment can be detrimental for the central nervous system (CNS) suggesting that neuroinflammation might represent an attractive pharmacological target to improve age-associated cognitive impairment. Hops is well known for its anxiolytic, anti-oxidant and anti-inflammatory properties. **Aims**: Here, we aim to determine whether a hop extract derived from a specific ecotype has anti-oxidant, neurogenic and anti-neuroinflammatory properties that may beneficially impact in aged CNS. **Methods**: Using *in vitro* stem cells- and neutrophils-based screening platforms we evaluate the neurogenic and the antioxidant potentials, respectively, of hop extracts obtained from different ecotypes. Next, aged rats (16 months old) were fed with selected hop extract for 42 days and adult neurogenesis as well as neuroinflammation were evaluated by immunohistochemistry. To assess neurogenesis, we administered bromodeoxyuridine (BrdU) for 5 days and the number of newly generated BrdU+NeuN+ neurons in the hippocampal dentate gyrus was determined 28 days after first BrdU injection. To asses neuroinflammation, we evaluated the number and morphology of Iba1-expressing microglia. **Results**: We found that the ecotype “RBX” displays the most robust anti-oxidant and neurogenic features and we have selected this for further *in vivo* experiments. Although results revealed no differences in the number of newly generated neurons, “RBX” induces a ramified microglia morphology accordingly with an anti-inflammatory phenotype. **Conclusions**: This study suggests that “RBX” exerts an antioxidant and anti-neuroinflammatory activities that might be beneficial for aged CNS, however, this needs to be further investigated.

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T14-035B

Development of cerebral organoids to study EVs-linked spreading of neuroinflammation in ALS-FTD.

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Cerebral organoids (COs) are the most up-to-date tool to model interaction between neurons and glia in the human central nervous system; as such COs are of great use for the study of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD): these are both fatal and irreversible diseases which are often co-diagnosed and clinically considered a spectrum. A pronounced neuroinflammation is often found in post-mortem tissues and is believed to be at the basis of ALS-FTD progression. The most common genetic cause of both these diseases is a G_4C_2 repetition present in an intronic region of the C9orf72 gene. Three toxic mechanisms are triggered by G_4C_2 expanded repeats in patients: formation of RNA foci, aberrant translation of dipeptide repeat proteins and haploinsufficiency/downregulation of the C9orf72 protein. C9orf72 exact function is still unknown but many evidence showed that C9orf72 might have a role in organelles biogenesis

maturation and function and indeed its absence hampers pathways such as autophagy or endocytosis. These pathways are interconnected also with production and release of extracellular vesicles (EVs). The term EVs refers to a broad class of spherical, double membrane structures produced and released by virtually any kind of cells. Recent evidence shows that neuro-glial interaction is tightly linked to mutual EVs exchange. We thus decided to study whether ALS-FTD related C9orf72 haploinsufficiency causes dysregulated EVs production which might boost and sustain the neuroinflammatory cycle which is at the basis of ALS-FTD progression. COs were established using control human induced pluripotent stem cells (hiPSCs) following a modification of the Lancaster protocol, which allows a better maturation of mesoderm layer and thus microglia development. By immunofluorescence we checked the correct and proper maturation of neurons using both markers of early neuronal maturation (such as Tbr2) and full neuronal maturation (Tuj1, Map2, Tbr1, NFL-H, CTIP). By real time qPCR we observed the increase over time of neuronal markers paralleled by the decrease of staminal markers. *Gfap* transcript levels start raising by day 30 of maturation and by day 60 we were able to detect astrocytes by immunofluorescence. We also isolated EVs in the COs culture media by ultracentrifugation at 100,000xg and characterized them by WB and NTA. Having successfully developed COs systems, the next step is generate a new hiPSCs C9-KO by employing CRISPR/Cas9 based gene editing in order to compare EVs production and content as well as astrocytes and microglia activation.

T14-036B

The interaction of activation state, sex and stage of development in *ex vivo* microglia

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Background: Resident microglia are present early in brain development, and undergo programmed maturation. Irrespective of their stage in the developmental cycle, microglia respond to abnormal conditions that can result in injury. However, it is not known if the transition from immature to mature microglia alters the response to inflammatory stimuli, or if these conditions also drive changes in key developmental markers. This is important for understanding the impact of stimuli at different stages of development, and in identifying immunomodulatory therapies useful in early and later life.

Methods: We isolated CD11B+ microglia from the postnatal day (P)3 or adult Swiss White mouse forebrain using saline perfusion then magnetic-activated cell sorting (Miltenyi Biotec). Microglia were isolated from males and females separately. P3 and adult CD11B+ microglia were plated at 30,000 cells/mm^2, and after 24h were then exposed for 18 h to interleukin 1 beta (IL1b, 25ng/mL) and interferon-gamma (IFNg, 50ng/mL); or, to interleukin-4 (IL4, 50ng/mL); or, to the equivalent volume of excipient (1uL, PBS). Cells were stained using immunohistochemistry for iNOS and COX2 for pro-inflammatory-related activation; IGF1, IL4 and ARG1 for anti-inflammatory-related activation; IL1RA for immunomodulatory-related activation; CRYBB1 for pre-microglial development; and CD14 for adult microglial development. Area coverage measurements were normalised to the total DAPI+ staining (Image J).

Findings: Activation with IL1b + IFNg or IL4 induced the expected changes in most markers of activation state but at P3 in the male, IGF1 and IL1RA expression was decreased by IL1b and increased by IL4, but not in microglia from female mice (both n=4). Similarly, iNOS and COX2 staining in adult male microglia increased and decreased following exposure to ILIb/IFNg and decreased IL4 respectively, but not in female adults. Expression of iNOS, COX2 and IGF1 was comparable between P3 and adult microglia under all conditions, but P3 microglia had lower

levels of Arg1, IL1RA and IL4 staining compared to adults. As expected, levels of the pre-microglia marker CRYBBI were higher in P3 (n=6) than in adult (n=3) mice, and CD14 expression was higher in the adult than at P3. Preliminary analysis indicates that IL1b/IFNg exposure at P3 reduces the expression of CRYBB1 in microglia from male but not female mice, but increases CD14 similarly in both sexes.

Interpretation: These studies show important sex and stage of development effects of responses to common activators of microglia and suggest that we need to explore further how activation itself impacts development.

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T14-037B

A novel anti-inflammatory pathway in murine microglia

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The Drosophila Glide/Gcm transcription factor is expressed and required in the differentiation of glia, the cells that act as the resident scavengers of the nervous system. The same factor is also necessary in the fly hemocytes, the scavenger cells that act outside the nervous system. The expression and requirement for Glide/Gcm in cells of immune nature as well as its anti-inflammatory role called for its evolutionary conservation in microglia, the resident scavenger cells of the vertebrate nervous system that have a large impact on neural development and on CNS pathologies.

Of the two Gcm murine orthologs, we found that mGcm2 is expressed in the brain, in particular it is present in a microglial subpopulation of old animals. These microglial cells present inflammatory features, suggesting that mGcm2 acts as a protective or a promoting factor in the inflammatory response.

To study the role of mGcm2 in microglia, we created a conditional knock-out mouse model. Brains from different age groups were analysed for their microglial phenotype. The results revealed that the lack of mGcm2 does not affect viability and that it leads to an enhanced inflammatory phenotype in old animals.

Our data hence highlight a novel molecular pathway controlling the inflammatory response and show that Gcm has an evolutionarily conserved anti-inflammatory role from flies to mammals.

T14-038C

In vitro model for inflammatory activation of human iPSC-derived astrocytes and microglia in multiple sclerosis

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Multiple sclerosis (MS) is a demyelinating and neuroinflammatory disease of the central nervous system (CNS) eventually leading to disability in young adults. Development of effective treatment options targeting the inflammation in progressive disease state have been largely unsuccessful. MS lesions are characterized by presence of activated astrocytes and microglia at the lesion rim and their pro-inflammatory phenotype has been linked to worsened disease progression. Neuroimmune interaction of glial cells partake in MS disease processes and may lead to enhanced immune responses, disturbed metabolism, loss of trophic support and axonal injury. Human cell -derived models are seen as a promising approach to reveal species-specific information about molecular mechanism underlying disease pathogenesis. Engineered *in vitro* platforms combining technologies of stem cell biology and microfluidics are utilized to model human astrocyte and microglia crosstalk and their role in pathology. Here, we studied human induced pluripotent stem cell (hiPSC) lines from healthy controls and patients suffering from MS. We induced a reactive phenotype in astrocytes and microglia and characterized their inflammatory phenotype. To mimic MS inflammatory milieu, we treated astrocytes with inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β and microglia with lipopolysaccharide (LPS). Cytokine-treated astrocytes experienced a change in morphology known as hallmark of astrogliosis and showed increased gene and protein expression of common inflammatory factors. Moreover, microglia express cell type specific markers and activation induced secretion of inflammatory mediators. Finally, we engineered a novel microfluidic device for studying microglia and astrocyte interaction in controlled, compartmentalized culture environment. Our model allowed study of microglia chemotaxis and recruitment by astrocytes after inflammatory stimulation. In conclusion, an *in vitro* model encompassing microglia, astrocytes and inflammatory component was developed that will serve to study glia-interactions and drug responses in MS disease.

T14-039C

Retinal microglia analysis in an early aging stage in healthy mice

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PURPOSE: To quantify the different morphological signs of microglial activation, as well as the analysis of P2RY12, MHC-II and CD68 expression in 15-month-old (aged naïve) mice, which is an early stage of aging, compared to 3-month-old mice (young naïve).

METHODS: Two groups of albino Swiss mice, young naïve (n=6) and aged naïve (n=6) were analyzed. Retinal whole-mounts were immunolabeled with: **1)** anti Iba-1 to quantify 1a) Iba-1 + cell number (Ibacn) in outer segments (OS), outer plexiform layer (OPL) and inner plexiform layer (IPL); 1b) area of retina occupied by Iba-1 + cells (Iba-1 RA) in the nerve fiber layer- ganglion cell layer (NFL-GCL); 1c) cell body area of Iba-1+ cells (CbIbac) in OPL, IPL and NFL-GCL; 1d) arbor area of Iba-1+ cells (AAIbac) in OPL and IPL; 1e) number of vertical processes of Iba-1+ cells (VPIbac) beetween the OS and OPL. **2)** anti-P2RY12 to identify resident microglia, anti-CD68 for phagocytic activity and anti-MHC-II as a microglial activation marker.
RESULTS: In aged naïve compared to young naïve the quantification of morphological changes showed: i) a non-significant increase in the Nibac in OS; ii) a non-significant decrease in the AAibac in OPL; iii) a significant increase in the Cbibac in OPL, IPL and NFL-GCL and iv) a significant increase in the VPibac. When we analyzed the expression of P2RY12, CD68 and MHC-II we observed: i) in young naïve all Iba-1+ cells were P2RY12+, except perivascular and dendritic cells, but in the aged naïve some cells were Iba-1+/P2RY12-; ii) in aged naïve numerous amoeboid-like CD68+ cells were found while in the young naïve the expression of CD68 practically was absent, and iii) in both young naïve and aged naïve no expression of MHC-II was observed.
CONCLUSION: In aged mice (15-month-old), the microglial cells shown morphological changes and in the expression of P2RY12, CD68 and MHC-II compared to young adult mice. These signs of microglial activation showed in a non-pathological state could influence age-related retinal diseases.

T14-040C

An investigation into the inflammatory function of oligodendrocyte lineage cells in mouse models of multiple sclerosis

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Multiple Sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system. Lymphocytes are necessary for the establishment and perpetuation of MS. Mechanisms of continued activation, clonal expansion, and maintenance of effector and memory lymphocytes in CNS compartments are not well understood. Neuropathological examination into the cellular composition of MS lesions establishes an overlap of inflammatory processes and repair mechanisms of oligodendrocyte lineage cells. Recruitment of OPCs to demyelinated lesions temporally and spatially overlap with the persistence of inflammatory infiltrates. OPCs can promptly respond to sites of injury responding to inflammatory cues. Oligodendrocyte lineage cells beyond the progenitor stage also have inflammatory functions. Oligodendrocyte lineage cells interact with CD4+ and CD8+ T-cells through antigen presentation on MHC class I/II molecules. Here we further interrogate the inflammatory function of OPCs/OLs (iOPCs/iOLs) induced by EAE driven CNS inflammation. iOPCs/iOLs upregulate surface molecule transcripts involved in canonical lymphocyte cell-cell interactions. Canonical PDL1 signaling is an immune checkpoint, acting through interaction with PD1 expressing lymphocytes. OPCs and oligodendrocytes upregulate *Cd274* transcripts in response to IFNγ, *in vitro,* and EAE, *in vivo*. Interestingly, iOPC/iOLs can express *Cd274* in combination with or independent of MHC class I/II expression, suggesting heterogeneity in their inflammatory response. Functional surface expression of PDL1 on iOPCs/iOLs was confirmed by flow cytometry analysis. Further, interrogation at the chromatin level through single-cell ATAC-Seq (Assay for Transposase-Accessible Chromatin) demonstrates increased chromatin accessibility at the *Cd274* locus iniOPCs/iOLs induced with IFNγ or

EAE compared to controls. Therefore, we hypothesize that iOPCs/iOLs may have a role in inflammatory regulation via their *Cd274*/PDL1 expression. Ongoing and future experimentation involves examining the mechanisms and consequences of iOPC/iOL Cd274/PDL1 expression by utilizing *in vitro* and *in vivo* methodologies.

T14-041C

Microglial activation in an experimental mouse glaucoma model at aging early stage

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PURPOSE: To quantify microglial cell activation sings, and P2RY12, MHC-II and CD68 expression in 15-month old mice compared to young adult mice, after unilateral laser-induced ocular hypertension (OHT).
METHODS: Four groups of albino Swiss mice; young naïve (n=6), aged naïve (n=6), young OHT (YG) (n=6) and aged OHT (AG) (n=6) groups were analyzed. In OHT groups, both OHT eyes and contralateral eyes were studied. Retinal whole-mounts were immunolabeled with: **1)** anti Iba-1 to quantify 1a) Iba-1 + cell number (Ibacn) in outer segments (OS), outer plexiform layer (OPL) and inner plexiform layer (IPL); 1b) area of retina occupied by Iba-1 + cells (Iba-1 RA) in the nerve fiber layer- ganglion cell layer (NFL-GCL); 1c) cell body area of Iba-1+ cells (CbIbac) in OPL, IPL and NFL-GCL; 1d) arbor area of Iba-1+ cells (AAIbac) in OPL and IPL; and 1c) vertical processes number of Iba-1+ cells (VPIbac) between OS and OPL. **2)** anti-P2RY12 (resident microglia), anti-CD68 (phagocytic activity) and anti-MHC-II (microglial activation marker).
RESULTS: Comparison of YG and AG with their respective naïve groups in both OHT eyes and contralateral eyes showed a significant increase of Ibacn, Iba-1 RA, CbIbac and VPIbac and decrease of AAIbac, more pronounced in OHT eyes. Comparison between YG and AG showed: i) AG vs. YG in OHT eyes, a significant decrease of Iba-1 RA and VPIbac; ii) AG vs. YG in contralateral eyes, a significant increase of Ibacn (OS) and a significant decrease of CbIbac and AAIbac (IPL). Regarding P2RY12, CD68 and MHC-II expression, we observed: i) in YG all Iba-1+ cells were P2RY12+, except perivascular and dendritic cells, but in AG most cells were Iba-1+/P2RY12-; ii) in AG and YG numerous amoeboid-like CD68+ cells were found but in AG, ramified appearance CD68+ cells were also observed, and iii) whereas in YG virtually all Iba-1+ cells were MHC-II+, in GA some Iba-1+ cells showed MHC-II+ expression.
CONCLUSION: The differences found in microglial cells both morphological and molecular in AG compared to YG show the possible aging implication in glaucoma.

T14-043C

Microglial metabolism is a pivotal factor in sexual dimorphism in Alzheimer's disease

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Age and sex are major risk factors in Alzheimer's disease (AD) with a higher incidence of the disease in females. Neuroinflammation, which is a hallmark of AD, contributes to disease pathogenesis and is inexorably linked with inappropriate microglial activation and neurodegeneration.

We investigated sex-related differences in microglial metabolism and activation in 18 month-old male vs female APP/PS1 mice.

Metabolism was assessed *ex vivo* in isolated microglia by measurement of glycolysis using Seahorse technology, metabolome analysis and multiplexed analysis of genes related to metabolism. Nucleo-cytoplasmic translocation of PFKFB3, a key glycolysis regulator, was assessed by immunocytochemistry. Microglial activation was appraised by analysis of morphology and CD68 expression by confocal microscopy in brain sections.

Microglia from female APP/PS1 mice were more glycolytic than microglia from males. This was mirrored by changes in abundance of lactate. Gene expression of glycolytic enzymes *Gapdh, Pgk1* and *Pgam1* significantly upregulated in microglia from female APP/PS1 mice compared with males and was accompanied by increased cytosolic PFKFB3 staining, indicating preferential enzyme activation, consistent with the glycolytic signature. Activation of microglia reflected by CD68 mRNA levels, CD68 immunostaining and rod-shaped morphology, was significantly greater in females than males.

Here we show that microglia from 18 month-old female APP/PS1 mice present a more profound shift in metabolism towards glycolysis accompanied by inflammatory and phenotypic changes, compared with males. We propose that the sex-related differences in microglia are likely to explain, at least in part, the sexual dimorphism in AD.

T14-044C

Fatty acid and lipid signalling in glial cells

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Abstract

Background: Neuroinflammation has been implicated in neurodegenerative disorders such as Alzheimer's disease and involves the activation of glial cells—microglia and astrocytes. Glial cells release different pro- and anti-inflammatory cytokines to maintain homeostasis of the brain. As a result of pathogen-derived or host-derived damage stimuli, microglia adopt an activated phenotype that produces various factors affecting astrocytes and neurons.

Peroxisome proliferator-activated receptor (*PPAR)* agonists have been well defined as having anti-inflammatory and neuroprotective functions in the central nervous system. However, the anti-inflammatory and metabolic roles of *PPARs* and fatty acid binding protein (*FABPs)* which can deliver PPAR ligands to the nucleus are not as well defined in glial cells as they are in other cell types.

Aims: The aim of this study was to fill a gap in the understanding of the expression of *PPARs*, *FABP5, FABP7 and FABP8* in glial cells. The study also evaluated different *in vitro* microglia models and the effect of different stimuli on microglia during development to understand the role of glial cells in neuroinflammation.

Method: Adult male Sprague-Dawley rats (P40) were used for adult microglia isolation, and both male and female neonate rats (P0-2) were used for neonatal microglia. Spinal and cortical microglia were cultured in serum-rich media by using the microglia shaking method or serum-free media using CD11b immunopanning. To trigger inflammation, microglia were incubated with 100 ng/ml LPS for 6 hrs or 100μM ATP for 3 hrs. Real-time qPCR was used for gene expression quantification. Microglial phagocytic ability was measured by assessing phagocytic uptake of fluorescent beads.

Results: Isolation of microglia using CD11b immunopanning produced a purer culture, and maintaining microglia in serum-free media appears to encourage a ramified morphology. *FABPs* and *PPARs* show a different level of expression in microglia dependent on developmental and regional status. No significant effect was found on gene expression after treatment with either ATP or LPS.

Conclusion: Our results suggest that *PPAR* and *FABP* expression in microglia is determined by developmental stage and anatomical location. Expression is unnafected by inflammatory stimuli. Further experiments will examine the effects of PPAR ligands upon inflammatory phenotype in microglia.

T14-045C

SCF+ astrocytes interact with c-Kit+ mast cells in the microenvironment of degenerating spinal motor neurons in ALS.

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Amyotrophic lateral sclerosis (ALS) pathology is characterized by degeneration of motor neurons, reactive astrocytosis, microgliosis and immune cell infiltration along the motor pathways that constitute a neurodegenerative microenvironment. Dysfunctional astrocytes contribute to ALS pathogenesis, motor neuron damage and accelerate disease progression. Mast cells (MC) can enter the central nervous system parenchyma in neurodegenerative diseases and have the potential to induce inflammation and disruption of the blood-spinal cord barrier (BSCB) via release of mediators such as cytokines, chemokines, histamine and proteases. However, the pathogenic significance and interaction between astrocytes and MC accumulating along the central motor pathways in ALS remain unclear. In the present study, we have identified that the cytokine SCF (stem cell factor) was upregulated in astrocytes in the lumbar spinal cord both in ALS patients and SOD1^{G93A} mice. SCF+ astrocytes closely interacted with MC expressing the SCF receptor c-Kit, which is known to signal MC migration and differentiation from progenitors. In addition, MC closely interacted with motor neuron and peri-neuronal capillaries and were associated

with peri-neuronal leakage of Evans blue dye, suggesting a pathogenic role of MC in disruption of the BSCB. Pharmacological inhibition of c-Kit with masitinib in SOD1^{G93A} mice reduced MC accumulation as well as BSCB disruption around the spinal motor neuron somas. Taken together, our results suggest a pathogenic mechanism triggered by astrocytes expressing SCF that leads to MC infiltration in the ALS spinal cord, which can be prevented pharmacologically.

T14-046C

Evaluation of myelin phagocytosis by microglia/macrophages in the nervous tissue using flow cytometry

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Determination of microglial phagocytosis of myelin has acquired importance in the study of demyelinating diseases. One strategy to determine microglial phagocytosis capacity consists in assaying microglia with fluorescently labelled myelin; however, most approaches are performed in cell culture, where microglia usually show important phenotypic differences compared to *in vivo* conditions. In this protocol, we describe an adapted flow cytometry protocol to assay myelin phagocytosis by microglia obtained directly from *in vivo* CNS tissue. Key steps for a first analysis of phagocytic microglia are provided. Additionally, we describe how to fluorescently label myelin using a pH-sensitive tag pHrodo™ Green STP ester.

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T14-047C

Müller glia-supported optic nerve regeneration in the adult zebrafish is triggered by infiltrating macrophages or microglia depletion

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Neurodegenerative diseases as well as ischemic and traumatic central nervous system (CNS) insults, importantly contribute to the most devastating and costly neurological disorders of our society. These deteriorations, characterized by progressive neuronal loss, eventually lead to functional impairment in the adult mammalian CNS and are often even fatal, due to the very limited regenerative potential of the CNS of adult mammals to replace lost neurons (de novo neurogenesis), or - the focus of our study - to repair damaged axons (axonal regeneration).

Stimulating and redirecting neuroinflammation has recently been put forward as an important approach to induce axonal regeneration, but it remains elusive how inflammatory processes and CNS repair are intertwined. To gain

more insight into these interactions, we investigated how immunomodulation affects the regenerative outcome after optic nerve crush (ONC) in the adult zebrafish, a versatile animal model with robust spontaneous regenerative capacity in the CNS.

Firstly, using flow cytometry and immunohistochemistry we observed that inducing intraocular inflammation using zymosan resulted in an acute inflammatory response, characterized by an increased infiltration and proliferation of innate blood-borne immune cells, reactivation of Müller glia and an altered retinal cytokine expression. Strikingly, we showed that the inflammatory stimulus also accelerates tectal reinnervation after optic nerve injury in zebrafish.

Secondly, we determined that acute depletion of both microglia and macrophages in the retina, using pharmacological treatments with both the CSF1R inhibitor PLX3397 and clodronate liposomes, compromised optic nerve regeneration. Moreover, we observed that *csf1ra/b* double mutant fish, lacking microglia in both retina and brain, displayed accelerated RGC axonal regrowth after ONC, which was accompanied with unusual Müller glia proliferative gliosis. Taken together, an influx of innate immune cells after inflammatory stimulation, as well as depletion of microglia, results in improved optic nerve regeneration in adult zebrafish. Strikingly in both cases, the accelerated axonal regeneration coincides with increased proliferative reactivation of Müller glia.Our results highlight the importance of altered glial cell interactions in the optic nerve regeneration process, and how altered direct (cell-to-cell contact) or indirect (cytokine signaling) interactions can reshape the overall regenerative response. Unraveling the relative contribution of the different cell types, as well as the signaling pathways involved, may pinpoint new targets to stimulate repair in the vertebrate CNS.

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T14-048C

Molecular mechanism of maternal immune activation induced parvalbumin positive interneuron deficit

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Maternal immune activation (MIA) is linked to the development of autism spectrum disorder in humans and rodents. Studies in rodents shows that MIA leads to decreased parvalbumin positive (PV+) interneuron number and autistic behavior changes, which is in part due to elevated maternal interleukin-17a level. However, the underlying molecular and cellular mechanisms in the fetal brain remain largely unknown. GPR56, a member of the adhesion G protein-coupled receptor family, plays a key role in brain development and its loss of function germline mutations leads to human brain malformation. GPR56 is highly expressed in microglia and is one of very few genes that help define yolk-sac derived ‘true’ microglia. Neuroinflammation in adults downregulates *Gpr56* expression in microglia. We hypothesize that GPR56 in fetal microglia mediates MIA-induced PV+ interneuron deficits in offspring. Here, we show that MIA downregulates microglial *Gpr56* expression in fetal brains in an IL-17a-dependent manner.

Conditional knockout of *Gpr56* in microglia mimics MIA-induced PV+ interneuron defect. Furthermore, microglial GPR56 regulates progenitor proliferation in the medial ganglionic eminence at late neurogenesis. Importantly, genetic rescue of Gpr56 expression in microglia reverses PV+ interneuron deficit in MIA offspring. Taken together, our study results demonstrate that microglial GPR56 plays an important role in PV+ interneuron development and serves as a salient target of MIA-induced neurodevelopmental disorders.

T14-049C

Regulation of injury responses of human oligodendrocytes by the Integrated Stress Response.

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Background: Early multiple sclerosis lesions feature relative preservation of oligodendrocytes (OLs) but with withdrawal (“dying back”) of their myelin processes. With disease progression, there is loss of OLs. Contributors to this pathology include metabolic stress, as well as proinflammatory molecules and excitotoxins. The Integrated Stress Response (ISR) pathway is a mechanism that preserves cell energy by attenuating protein translation, either directly or by interaction with the mechanistic target of rapamycin (mTOR) pathway. Reduced protein synthesis could impact directly on the process retraction of OLs in MS. Constituents of the ISR can be detected in OLs in MS lesions (Mhaille, 2008).
Objectives: To determine if the ISR pathway participates in the process retraction and cell survival of human OLs (hOL) in response to metabolic, inflammatory, and excitotoxic insults.
Methods: We used human OLs isolated from surgically resected brain tissue samples to assess their *in vitro* functional and molecular properties in response to low glucose (LG), tumor necrosis factor a (TNFa) and glutamate. Cell death was evaluated by propidium iodide staining. Cell area was assessed using Imaris analysis software. Dissociated cultures of rat post-natal brains were used for comparison.
Results: Exposure of the hOLs *in vitro* to metabolic stress conditions (LG) resulted in process retraction without significant cell death at 24-48 hours. Non-apoptotic cell death developed over 4 to 6 days. In contrast rat OPCs showed significant apoptotic cell death within 12 to 24 hours. Microarray analysis of hOLs under LG versus optimal conditions at 48 hours showed significant upregulation of ISR markers associated with a down regulation of the mTOR pathway and an attenuation of the overall protein synthesis. Immunostaining for ATF4 and CHOP protein expression confirmed activation of the ISR pathway prior to any cell death. Treatment with ISR enhancer Sephin1 further reduced process area under LG conditions. Addition of ISR inhibitor ISRIB after 24 hours of LG condition resulted in recovery of process extension. Neither drug had a significant influence on cell death. In contrast to effects of LG, TNFa and glutamate both induced process retraction without significant cell death and without apparent activation of the ISR pathway.
Conclusion: ISRactivation, acting via regulation of protein synthesis, provides a mechanism whereby metabolic stress can contribute to the process retraction of human OLs, a feature of early MS lesions. Whether prolonged ISR activation continues to provide a putative protective response or contributes to cell death remains to be determined. Retraction induced by inflammatory/excitotoxin may represent distinct direct effects on cell processes.

T14-050C

In vivo imaging of oligodendrocyte injury in an NMO mouse model

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Neuromyelitis optica (NMO) is an autoimmune disease predominantly affecting spinal cord and optic nerve[1]. The majority of NMO patients have serum antibodies (IgG) against the water channel protein aquaporin 4 (AQP4), which in the CNS is expressed on astrocytic end-feet and ependymal cells[2, 3]. Despite this primary astrocytic target, demyelination is also prominent in AQP4-IgG+ NMO, and is regarded as 'secondary' to astrocyte loss[4]. However, the in vivo mechanisms by which targeting an astrocytic antigen can drive injury of other cell types, such as oligodendrocytes, remain unresolved.

We investigated early signs of oligodendrocyte damage in a mouse model of acute NMO-related pathology induced by spinal application of patient-derived AQP4-IgG and human complement followed by in vivo imaging[5]. Morphological assessment and calcium imaging of genetically labeled astrocytes and oligodendrocytes revealed the relative time-course of glial cell injury: Within an hour of AQP4-IgG application, intracellular calcium levels in astrocytes increased globally and membrane rupture swiftly followed as confirmed by uptake of a cell-impermeable nuclear dye and subsequent cellular fragmentation. Concurrent to the global astrocytic calcium rise, oligodendrocyte processes also showed calcium overload, which however reached the soma comparatively later (Fig. 1). In contrast to the pervasive and swift lytic cell death of astrocytes, only some oligodendrocytes were lost at later time points. While dye exclusion experiments negated overt membrane rupture, expression of human MAC-inhibitor protein CD59 on oligodendrocytes still protected these cells from secondary damage after AQP4-IgG–mediated astrocyte injury.

These results imply that oligodendrocyte pathology in NMO is not driven by the loss of astrocytes per se, but rather evolves from MAC-dependent 'bystander' targeting of oligodendrocytes as suggested by prior in vitro and fixed tissue observations[6]. At the same time, our dynamic observations suggest that despite the similar starting point of injury, the executive phase of cell injury might differ and could result in activation of distinct cell death pathways in the two major glial target cells of NMO.

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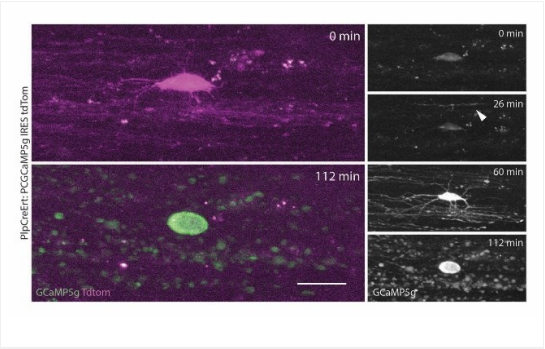

Figure 1. Oligodendrocyte injury in NMO mouse model
Morphological assessment and calcium imaging of genetically labeled oligodendrocytes revealed the relative time-course of AQP4-IgG-mediated glial cell injury. Oligodendrocytes showed calcium increase as early signs of cellular injury, which was followed by morphological changes during acute NMO lesion formation.

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T14-051C

Schwann Cell-derived Small Extracellular Vesicles Function as “TNFα Decoys” in Painful Peripheral Nerve Injury

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In Wallerian degeneration, various cytokines are expressed in a predictable time course and at defined levels to facilitate removal of damaged cellular debris and facilitate nerve regen-eration. Aberrations in cytokine expression may lead to failed functional regener-ation. Tumor necrosis factor (TNFa) is expressed by Schwann cells (SCs) early in the course of Wallerian degeneration and serves as a major regulator of SC physiology and gene

expression. We have shown that primary rat SCs in culture express abundant levels of TNF Receptor-2 (TNFR2) and lower levels of TNFR1. These SCs create and disperse small extracellular vesicles (EVs), which are highly enriched in TNFR1. We isolated EVs from cultures of rat SCs by sequential ultracentrifugation and authenticated these preparations by nanoparticle tracking analysis, electron microscopy, and immunoblotting for EV and SC biomarkers. TNFR1 constituted almost 2% of the total protein in SC-derived EVs. SC EV TNFR1 was active in binding TNFα. When added to SC cultures together with TNFα, SC-derived EVs blocked many of the anticipated effects of TNFα in SCs, including p38 MAPK phosphorylation (**Fig. 1**), secondary cytokine expression, and cell death. SC-derived EVs also were active when injected directly into sciatic nerves. In these studies, SC EVs prevented p38MAPK phosphorylation in res-ponse to TNFα *in vivo* and changes in sciatic nerve morphology resulting from inflammatory cytokine exposure. TNFα-induced tactile allodynia was significantly reduced when TNFα was injected into sciatic nerves together with SC EVs. These results demonstrate the ability of SC-derived EVs to display "TNFα decoy activity". By binding TNFα, SC EVs decrease the concentration of free TNFα available to interact with SCs and other cells in the injured peripheral nerve microenvironment. We propose that SC EVs may be important regulators of the cytokine milieu in Wallerian degeneration and neuropathic pain.

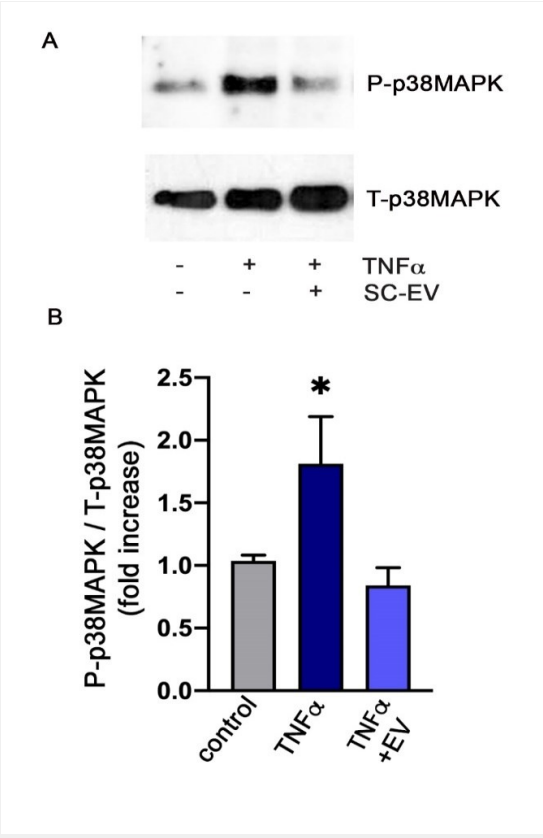

Figure 4. TNF induces proinflammatory cell-signaling that is blocked by SC EVs.
A) Representative immunoblots showing activation of p38MAPK by TNFa 500 pM) that was blocked with co-administration with SC EV (10 µg). (***B***) Densitometry analysis was performed to determine the relative levels of P-p38MAPK standardized against Total-p38MAPK mean ± s.e.m.; n=6 independent experiments, *$P<0.05$ comparing with the control group using a one-way ANOVA with a Tukey's *post hoc* test).

T14-052C

Cathepsin C Aggravates MOG_{35-55} induced Experimental Autoimmune Encephalomyelitis by promoting the differentiation of Th17 and Tfh cells

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Multiple sclerosis (MS) is a chronic autoimmune disease in the central nervous system (CNS) where both T cells and B cells are implicated in pathology. Experimental autoimmune encephalomyelitis (EAE) is the most employed model for MS, which is mainly mediated by Th1, Th17 and Tfh cells. Cathepsin C (CatC) is a cysteine exopeptidase which function as a key enzyme in the activation of granule serine proteases in immune cells through removing N-terminal pro-dipeptides from the zymogen forms of these proteases. In our previous study, we have demonstrated that CatC can promote microglia(MG) and macrophage(Mφ) toward M1 activation status and aggravate demyelination in EAE animal model, however, the mechanism involved in the process of EAE, namely how CatC takes effect on adaptive immune response, has not been elucidated. In present study, we used conditional overexpression of CatC gene in Iba-1 positive cell mice (CatCOE) to establish MOG_{35-55} induced EAE model. During immunization for 50 days, the clinical score was valuated every day, and flow cytometry analysis was performed to analyze the frequency of Th1, Th2, Th17, Tfh, Treg, plasma cell, MG/Mφ from spleen, draining lymph node and brain at 7^{th}, 10^{th}, 14^{th}, 23^{th} and 50^{th} day after immunization, respectively. We found that CatCOE significantly aggravated clinical scores during the onset and persistent stage in EAE model combined with significantly increased frequency of Tfh and Th17 cells in the spleen, draining lymph node and brain. Simultaneously, the mRNA expression of series factors such as IL-6, IL-23a, IL-12b, Bcl-6 and Rorc, which located on the upstream of Tfh and Th17 differentiation, was found upregulated in primary cultured MG cells and BV2 after exogenous MOG_{35-55} stimulation and in CatCOE mice brain and spinal cord after immunization. And on the downstream, the concentration of IL-21 and IL-17A, as well as the frequency of plasma cell and the level of MOG_{35-55}-specific IgG, was also found significantly increased. Furthermore, upregulated MHC-Ⅱ and CD86 expression was found in Mφ and MG in CatCOE mice after immunization. These results suggest that CatC may enhance MG and Mφ antigen presentation capability and product more IL-6, IL-21, IL-12b and IL-23a to promote Th17 and Tfh cells differentiation, in turn, to facilitate plasma cell and MOG35-55-specific IgG antibodies production to exacerbates the EAE progression.

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T14-053C

Detrimental role of type I interferon-activated microglia in Neuromyelitis Optica.

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Neuromyelitis optica (NMO) is an immune-mediated chronic inflammatory disease that manifestates with optic neuritis and myelitis. The characteristic feature of NMO is a presence of autoantibodies against water channel aquaporin 4 expressed in the central nervous system on astrocytes, which underlies astrocyte killing. There is no cure for NMO, and treatment with type I interferon (IFNI)- IFNβ is ineffective or even detrimental. We have previously shown that both NMO lesions and associated microglial activation were reduced in mice lacking the receptor for IFNβ. However the role of microglia in NMO is elusive. In this study we aimed to clarify the role of IFNβ as well as to assess the importance of microglia in experimental NMO. Treatment with IFNβ led to exacerbated pathology and further expansion of $CD11c^{+}$microglia subset. Importantly, depletion of microglia resulted in a suppression of astrocytopathy and decreased expression of IFNI signature genes. Taken together, our data show a detrimental role for IFNI-activated microglia in NMO and open new perspectives for microglia-targeted therapies

T14-054C

Enteric glia shape intestinal immune homeostasis and tissue responses to parasite infections

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Intestinal tissue homeostasis and response to infections is achieved by the integrated activity of multiple cell types such epithelial, immune and stromal cells, but the role of intrinsic neuroglia networks in these processes remain largely unknown. Here, we reveal pivotal roles of enteric glial cells (EGCs) in intestinal tissue homeostasis and repair following infectious challenge. High resolution transcriptomic analysis of EGCs from mice infected with the helminth *Heligmosomoides polygyrus (H. poly)* and patients with inflammatory bowel disease identified dynamic glia cell states linked to immune responses in the gut wall. We also demonstrate that EGC-specific ablation of IFN-γ-signalling results in activated inflammatory state of tunica muscularis and defective repair of tissue damage associated with helminth infections. Our studies reveal that IFN-γ as key regulator of glia function and demonstrate that the IFN-γ-EGC signaling axis is central to immune homeostasis and tissue repair in the mammalian gut.

T14-055D

Pro-resolving mediator lipoxin ATL reduces Aβ-induced oxidative stress and mitochondrial dysfunction in neurons and microglia in vitro

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Neuroinflammation is a key contributor to Alzheimer's disease (AD) progression. Acute activation of glial cells can enhance amyloid-β (Aβ) clearance and neuroprotection. However, when inflammation becomes chronic, glia turn to a dysfunctional phenotype, which leads to synaptic loss and neuronal death. Lipoxin LXA_4 is a lipid mediator with potent anti-inflammatory and pro-resolving activity. One of the main contributors to neuroinflammation is oxidative stress, and LXA_4 helps resolve this by interacting with reactive oxygen species (ROS) generating enzymes like NOX2. Besides, LXA_4 is reduced in CSF and hippocampus of AD patients, and it correlates with cognitive impairment. Taking all of this into account, we further studied the effects of lipoxin LXA_4 in Aβ-induced oxidative stress.

In order to do that, we used astrocyte, neuron and microglia primary cultures, as well as organotypic cultures. We used the metabolically stable LXA_4 analog aspirin-triggered lipoxin (ATL), in presence or absence of lipopolysaccharide (LPS) or Aβ oligomers. Boc2 peptide was used as an antagonist for LXA_4 receptors LXA1 and LXA2. We used fluorescent ROS detection probes CM-H2DCFDA and mitoSOX, and performed Seahorse analyses to measure mitochondrial function. We also used colorimetric assays to analyse superoxide dismutase activity in primary cultures, and propidium iodide to address cell death in organotypic slices.

Firstly, we found that ATL reduced LPS-induced ROS generation in astrocytes, neurons and microglia. ATL also diminished Aβ-induced ROS in neurons and microglia. We also observed a blockage of ROS reduction with Boc2, which confirmed that ATL was acting through its specific receptors ALX1 and ALX2. In addition to reducing general ROS production, ATL reduced Aβ-induced superoxide production in the mitochondria of neurons and microglia. Given that ATL has a positive effect on mitochondrial ROS, we analysed respiration in both neurons and microglia. We found that Aβ significantly reduced basal respiration and ATP production, and ATL was able to recover both parameters to control levels in these two cell types. Additionally, we found that ATL significantly increased the superoxide dismutase activity reduced by Aβ in neurons and microglia. Lastly, we found that ATL reduced Aβ-induced ROS generation and cell death in corticostriatal organotypic slices.

Overall, these results indicate that ATL could reduce Aβ-induced oxidative stress by reducing ROS production, recovering mitochondrial function and enhancing ROS elimination. Therefore, ATL treatment could be a potential therapeutic approach to address neuroinflammation in AD.

T14-056D

The deletion of microglia exacerbates anorexia induced by the food contaminant Deoxynivalenol

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Deoxynivalenol (DON), one of the most abundant trichothecenes found on cereals, has been implicated in mycotoxicosis in both humans and farm animals. DON-induced toxicity is characterized by reduced food intake and weight gain, diminished nutritional efficiency and immunologic effects. We previously shown that exposure to DON induced an inflammatory response by increasing the expression of pro-inflammatory cytokines within the brain. This central production of inflammatory cytokines was proposed to contribute to DON-induced anorexia. Microglia cells, the primary immune cells of the central nervous system, contribute to neuroinflammatory processes during diverse pathophysiological conditions. To date, the possible contribution of these cells to DON-induced brain inflammation and anorexia has not been evaluated. Here, we reported a microglia activation (increased IBA-1 reactivity) in response to anorectic DON doses administered by gavage. Interestingly, this microglial activation was observed while DON-induced anorexia was ongoing (i.e., 6h post DON administration). Next, we took advantage of pharmacological microglia deletion using a diet containing a CSF1R-inhibitor, referred to as PLX3397 (Plexxikon Inc.). Surprisingly, microglia depleted mice exhibited an increased sensitivity to DON since non-anorectic DON doses became toxic in PLX3397-treated mice. Similarly, inactivation of microglial cells by intraperitoneal administration of minocycline resulted in anorexia with dose ineffective in control mice. Moreover, low DON doses induced c-Fos expression within feeding behavior-associated structures in both PLX3397- and minocycline-treated while not in control mice. To exclude a possible alteration of gut permeability by PLX3397 or minocycline treatments that could have resulted in an increased DON absorption, we proceeded to DON intraperitoneal administration. As previously observed, PLX3397- or minocycline-treated mice exhibited an increased sensitivity to the anorectic action of DON. Altogether, these results strongly suggest that microglia cells, activated in response to DON absorption, limit the toxicity of this mycotoxin. While the precise mechanisms underlying this action remain to be characterized, this model could help address the broad question of microglia functions during environmental toxins exposure.

T14-057D

TREM2 promotes microglial reprogramming and resilience to subcortical white matter disease in a model of vascular cognitive impairment

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Cerebrovascular disease is a major contributor to subcortical white matter pathology, vascular cognitive impairment (VCI) and dementia. Although the precise pathophysiological mechanisms remain unclear, it is increasingly evident that inflammatory and cerebrovascular processes interact. White matter is particularly vulnerable to vascular insults and is prone to more intense inflammatory reactions than grey matter due to inter-regional heterogeneity in vascular anatomy and microglial phenotype. Thus, regulators of microglial homeostasis and reactivity may be critical to the development and progression of chronic cerebrovascular disease and its cognitive consequences. To

investigate this, we used a mouse model of VCI induced by bilateral carotid artery stenosis (BCAS) and determined the impact of genetically deleting the microglial immunoreceptor triggering receptor expressed on myeloid cells 2 (TREM2), a key regulator of microglial homeostatic and reactive functions. Pathological assessment identified diffuse white matter injury associated with reactive gliosis 1 month following BCAS. Interestingly, TREM2 deficiency attenuated microglial density in areas of subcortical white matter damage and blunted expression of markers associated with reactivity (CD45). Importantly, assessment of myelin integrity demonstrated that blunted microglial reactivity was associated with greater white matter damage in Trem2-/- mice. Transcriptomic analysis of microglia isolated from white matter 1 month after BCAS revealed induction of gene expression modules associated with inflammation, chemotaxis, lysosomal function, lipid processing and metabolic reprogramming were heavily attenuated in Trem2-/- mice. Overall, these data suggest TREM2-regulated microglial reactivity contributes to white matter resilience in the context of chronic cerebrovascular dysfunction. Further studies will define mechanisms conferring TREM2-mediated resilience to cerebrovascular dysfunction and include in vitro and in vivo models to assess the importance of TREM2-mediated myelin clearance and metabolic adaptations during chronic cerebrovascular dysfunction.

T14-058D

Motoneuron deafferentation and neuroinflammation in association with aging in the spinal cord of C57BL/6J mice.

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Aging is accompanied by functional and structural alterations in the neuromuscular system. In the present study, we simultaneously analyzed changes in afferent synaptic inputs to motoneurons (MNs) and in their surrounding glial cells occurring with aging in the mouse spinal cord.
C57BL/6J adult (4 months) and old (30 months) mice were used. Immunofluorescent labeling was performed using antibodies against Iba1 and GFAP (for microglia and astroglia, respectively). Additionally, antibodies against Mac-2 and CD206 (for pro-inflammatory M1 and neuroprotective M2 microglial phenotypes, respectively), and 14-3-3 and S100β (to label A1 and A2 astroglial phenotypes, respectively), were also used. Excitatory (cholinergic and glutamatergic) synaptic terminals on MNs were also examined by using anti-vesicular acetylcholine transported (VAChT) and vesicular glutamate transporter 1 and 2 (VGluT1 and VGluT2) antibodies.
We found that aging is associated to prominent microgliosis and astrogliosis (~93% and ~100% increase vs. adults, respectively) around MNs, with significant increased density of pro-inflammatory M1 microglial and A1 astroglial phenotypes (25-fold and 4-fold increase, respectively), and reduced proportion neuroprotective M2 microglia and A2 astroglia. Moreover, aged MNs were depleted of cholinergic and glutamatergic inputs (~40% and ~45%, respectively), suggestive of age-associated alterations in MN excitability and firing[1].

To assess the activated state of microglial cells, we used an antibody against cluster differentiation 68 protein (CD68), a member of the lysosomal/endosomal-associated membrane glycoprotein (LAMP) that regulates phagocytosis in macrophage lineage cells. Double immunocytochemistry for CD68 and Iba1 in spinal cords of adult and old mice revealed that, virtually all CD68-positive profiles are located inside Iba1-immunolabeled cells. Compared with adult spinal cords, those of aged mice exhibited a significant increase in CD68-expression in microglial cells located around MNs.

Overall, these results indicate that aging is accompanied with overt sings of spinal cord gliosis which entails a marked increase in the microglial and astroglial pro-inflammatory phenotypes (M1 and A1, respectively) to the detriment of anti-inflammatory and neuroprotective (M2 and A2) glial subpopulations. Age-related gliosis is strongly associated to the loss of excitatory synaptic inputs to MNs. Further work is necessary to examine the relevance of gliosis in MN deafferentation occurring with aging and the impact of both processes in motor-activity defects found in the elderly.

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T14-059D

Myelin as a risk factor in autoimmune-mediated injury

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Multiple sclerosis (MS) is the most common cause of non-traumatic disability in young adults which is ultimately determined by the degree of axonal damage. In animal models, focal axonal injury is often linked to demyelination and found in areas devoid of myelin. We thought to test this prevailing concept by investigating the interdependence between the myelination status and axonal injury in mouse models of demyelination as well as multiple sclerosis. We detected a multitude of damaged axons with intact myelin sheaths in both MS and its model experimental autoimmune encephalomyelitis (EAE). In EAE, we could also show that axonal swellings indeed form in myelinated axons and subsequently resolve over time. However, irreversibly damaged axoplasm-dense nerve fibers accumulate with disease progression and are solely found in myelinated axons. This prompted us to ask whether myelinated axons are at special risk of degeneration. To answer this question, we made use of a novel mouse line, termed hMBP (myelin basic protein hypomorph), which is characterized by an increased number of amyelinated axons. Indeed, in EAE spinal cord lesions of hMBP animals, the number of damaged axons was reduced and, importantly, still confined to myelinated axons. Additionally, the overall clinical score was significantly reduced in these animals. This points towards a novel role of myelinating oligodendrocytes in conveying damage to axons, possibly by the inability of providing metabolic support. Understanding the mechanisms behind this type of secondary axonal injury is crucial for developing oligodendrocyte-targeted therapies.

T14-060D

In vivo and *in vitro* effects of bilirubin on NLRP3 inflammasome activation

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Neonatal hyperbilirubinemia is a common situation characterized by increased unconjugated bilirubin (UCB) levels. UCB is mostly bound to albumin, but occasionally may be present as free bilirubin in the central nervous system, which may cause injury to developing nervous system. Hyperbilirubinemia may lead to inflammation and excitotoxicity. Exposure to high levels of UCB may cause irreversible cellular damage. The molecular mechanism of bilirubin neurotoxicity has not yet been fully illuminated.

Neuroinflammation takes part in bilirubin-related neurological dysfunction (BIND) pathogenesis. Although the protective effect of low dose, chronic neuroinflammation is considered a critical risk factor for central nervous system damage in the neonatal period. Inflammasome activation is one of the immuno-reactive mechanisms of the innate immune system and differs from classical inflammatory responses. Membrane-bound or cytosolic receptors, such as NOD-like receptors (NLRs) are responsible for inflammasome activation. The aim of this study is to investigate the effect of bilirubin in inflammasome activation in *in vitro* and *in vivo* examinations, and to decide if the inflammatory activation depends on NLRP3.

Thus, N9 microglia cell line have been examined for inflammasome activation after the application of bilirubin w/wo LPS . Bilirubin plus LPS administration has increased IL-1β secretion and NLRP3, IL-1β , IL-18 mRNA level. In addition, NLRP3 protein expression, cleaved caspase-1 and cleaved IL-1β protein level has been upregulated. *In vivo* part of study includes the determination of whether the bilirubin activates the NLRP3 inflammasome markers in brain tissue. Hyperbilirubinemia model was created injection of bilirubin into cisterna magna of C57BL/6 mouse. Significant increase in NLRP3 and IL-1β mRNA expression level were determined by bilirubin administration. In conclusion, examining the relationship between inflammasome and bilirubin will result in the identification of molecular pathways of bilirubin neurotoxicity and might help new treatment strategies.

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T14-061D

Induction of hypoxia related programs in astrocytes exacerbates Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS). Tissue damage and in particular neuro-axonal degeneration are the correlate of irreversible disability in patients, but mechanisms thereof are still poorly defined. Previous studies have reported upregulation of reactive oxygen species (ROS) and nitirc oxide (NO) in MS lesions. Furthermore, structural and functional damage of mitochondria have been described. Tissue hypoxia seemed one plausible explanation for these findings. Paradoxically, treatment studies with both hyperoxygenated and chronic mild hypoxia have shown beneficial effects in the animal model experimental autoimmune encephalomyelitis (EAE). In our study we analyzed whether the astrocyte-specific activation of hypoxia related pathways after onset of EAE would improve the clinical outcome. Here, we made use of a mouse strain with a tamoxifen inducible astrocyte-specific knock out of the oxygen sensors and hypoxia negative regulators prolyl hydroxylases (PHD) 2 and 3 (namely Aldh1l1-cre^{ERT2}; Phd2$^{fl/fl}$; Phd3$^{fl/fl}$). In these mice, PHD2/3 knockout leads to a reduced rate of ubiquitination of HIF1a and HIF2a, which strongly induces their dependent genes in astrocytes. We took a therapeutic approach by inducing the PHD2/3 knockout after onset of clinical signs. Surprisingly, during the 30 days post disease onset and tamoxifen treatment a significant worsening of the EAE score, weight loss and neurological fitness (measured by Rotarod) was observed in the PHD2/3 knockout group. FACS analysis of cells isolated from spinal cord revealed significantly increased numbers of inflammatory immune cells. In parallel, Aldh1l1-specific mRNA pulldown was performed by translating ribosome affinity purification (TRAP) followed by bulk RNA-seq. These data revealed that astrocytes of both EAE groups expressed pan reactive, A1 and A2 specific genes in contrast to controls. A deeper comparison of ko vs. wt highlighted 60 significantly differentially expressed genes (DEGs), which where furthermore significantly enriched in distinct pathways. We conclude that induction of hypoxia response genes in astrocytes exacerbates clinical EAE by fueling the inflammatory part of the disease.

T14-062D

Basal autophagy inhibition in microglia diminishes phagocytosis of apoptotic cells and microglial survival

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Autophagy is the cellular process whereby cytoplasmic constituents such as long-lived proteins and damaged organelles are delivered to the lysosome for degradation. Autophagy is basally active in virtually all cell types, wherein it functions as a cellular quality control checkpoint to promote cellular fitness and survival. However, the role of basal autophagy in microglia, the brain resident macrophages, has started to emerge only recently. In this study, we have evaluated the effect of basal autophagy inhibition in microglial phagocytic function and survival. To monitor the autophagic activity of microglia, we first developed a 2-step model to separately assess autophagosome formation and degradation in microglia using conventional LC3 Western blot assays. Using this

model, we confirmed that after treatment with the unc-like kinase1/2 (ULK1/2) inhibitor MRT68921, which inhibits the autophagy pre-initiation complex, reduced both autophagosome formation and degradation proportionally in primary microglia, leading to a reduced turnover ratio of autophagosomes and an inhibition of basal autophagy. Next, we assessed the effects of basal autophagy inhibition in microglial phagocytosis of apoptotic cells and survival using pharmacological in vitro and genetic in vivo approaches. In vitro, MRT68921 impaired microglial phagocytosis of apoptotic cells at low concentrations that did not induce microglial death. However, high concentrations of MRT68921 did induce significant microglial death, suggesting that basal autophagy disruption is critical for microglial survival. In vivo, constitutive deletion of autophagy-related genes such as ATG4B, also disrupted microglial phagocytosis and decreased microglial survival in the neurogenic niche of the hippocampus, where newborn neurons constantly undergo apoptosis and are rapidly engulfed by microglia. We are now extending our analysis to other microglia-specific autophagy-deficient mouse models such as ULK1 or BECN1. In conclusion, our results indicate that basal autophagy shapes microglial fitness regulating their function and survival.

T14-063D

Disease severity affects NG2 cell proliferation during the disease course of multiple sclerosis

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Inflammation-induced demyelination of the central nervous system (CNS) is the main histopathological hallmark in Multiple Sclerosis (MS) and its animal model, Experimental Autoimmune Encephalomyelitis (EAE). Improving remyelination in MS is essential for promoting tissue repair and alleviating the associated neurological impairment. In this sense, we recently reported that myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immature myeloid cells with immunosuppressive activity, are involved in oligodendocyte precursor cell (OPC) survival, proliferation and differentiation, being the osteopontin the main MDSC effector. Furthermore, our group also observed that the abundance of MDSCs in the peripheral blood at onset of EAE symptoms is inversely correlated with the severity of the clinical course and the degree of demyelination. In addition, we saw a direct correlation between the abundance of MDSCs in the peripheral blood and the density of Arg-I$^+$ (MDSCs) and NG2$^+$-cells (OPCs) associated to areas of de-(re)myelination in the spinal cord at the peak of the disease, pointing to MDSCs as a useful bioindicator not only for the future clinical course severity but also for a better capacity of CNS repair.

In the current work, we give more insights in the proliferative ability of NG2$^+$-cells and MDSCs in the spinal cord of EAE mice during both the inflammatory and the immunomodulatory phases of the clinical course. On the one hand, we analyze the distribution of MDSCs and proliferating NG2$^+$-cells in the spinal cord of EAE mice by BrdU injection during the inflammatory phase. Firstly, we observe that the severity of the clinical course seems to determine the cellular distribution of the CNS, showing a higher density of MDSCs, proliferating NG2$^+$cells and CC1$^+$-oligodendrocytes in EAE mice with a milder clinical course. Remarkably, the peripheral load of MDSCs at the onset of the symptoms directly correlates with the density of proliferating NG2$^+$ cells at the peak of the disease. On the other hand, with the goal of investigating the MDSC and the NG2$^+$-cell distribution during the immunomodulatory phase, we analyze not only the cell density at the end of the recovery but also its correlation with the peripheral load of MDSCs at the peak. Furthermore, we deepen in the study of MDSC and NG2$^+$-cell proliferation in the spinal cord of EAE mice during both inflammatory and immunomodulatory phases by BrdU and EdU injections at the

onset and at the peak, respectively. In sum, our results shed more light on the factors involved in the variability of the clinical course disease and the role of MDSC as a bioindicator of a CNS prone to succeed in repair in the context of MS.

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T14-064D

Effects on the hippocampal microglia after acute treatment of a psychological stressor associated with depressive-like behaviours

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Stressful life events may have a negative impact on mental health compromising people's well-being, so knowing the neurobiological changes that occur after psychosocial stressors can have an impact on overall health. However, the neurobiological mechanisms responsible for the negative effects are not known in detail, and the initial changes that take place after the initiation of a stress protocol are much less well understood. Hippocampus constitutes a target structure of the adverse effects of stress. Among the possible mechanisms involved, the response of microglia to stress is receiving increasing interest. For this reason, after 1 and 24 hours of submitted C57BL/6J mice to acute and intense stress procedure denominated WIRS (water immersion restraint stress), the microglial response were analysed using a set of morphofunctional parameters. Then, the levels of the cytokines: IL-6, IFN-gamma and TNF-alpha were measured. Furthermore, a complementary proteomic analysis based on the principle of mass spectrometry was carried out. Results reveal that acute stress increased the number of microglia and induced microglial morphofunctional changes. Regarding cytokines, acute stress only increased IL-6 levels, which remained elevated at 24 h. Proteomic analysis, over time (in 24 h post-stress), showed an increase in proteins associated with the intracellular calcium metabolism. These findings suggest a neuroinflammatory response after acute stress observed at one hour after the application of the WIRS protocol and maintained at least 24 hours after the end of the stressor.

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T14-065D

Chemically functionalized single-walled carbon nanotubes alter the cytokine profile of the stretch-injured cultured mouse astrocytes

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Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide and represents a significant public health problem. In the central nervous system, astrocytes act as regulators of the brain environment and have a role in the synaptic plasticity and the reorganization of neural circuits following TBI. Carbon nanotubes represent promising nano candidates in treating brain diseases and injuries. They have excellent physicochemical properties, and the ability to interact with neurons, neuronal circuits, and astrocytes. The aim of our study was to test the effects of the application of chemically functionalized single-walled carbon nanotubes (SWCNTs) on the release of cytokines from the primary mouse astrocytes exposed to severe *in vitro* TBI.

All research was performed on the primary astrocyte cultures that were grown on 6-well plates with silastic membranes. Severe stretch-injury was induced by controlled release of pressurized nitrogen, with a peak pressure of 3.8-4.2 PSI. One-hour post-stretch, SWCNTs, or vehicle were applied and 23 h later cell media were collected for the determination of the cytokine profile. Non-injured, vehicle-treated astrocytes were used as the control group. For the measurement of the cytokine levels, we utilized the Mouse Cytokine Array C3 kit (RayBiotech, Inc.), which can detect changes in expression of 62 different proteins with high sensitivity (pg/ml).

Application of the chemically-functionalized SWCNTs to the injured cells caused an increase in the release of the axl, BLC, GM-CSF, IL3 Rb, IL4, IL5, IL6, and IL10 in regards to the levels determined in the cell media from the non-injured cells. Astrocytes treated with the investigated nanomaterial also released higher amounts of axl, BLC, G-CSF, GM-CSF, IL4, IL5, IL6, IL9, and IL10 compared to the levels determined in the samples from the injured, vehicle-treated cells.Our results showed that the investigated SWCNTs cause changes in the cytokine release from the cultured astrocytes exposed to severe *in vitro* TBI pointing to the possible mechanism of their effects in the injured neural tissue.

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T14-066D

iPSC-derived Microglia for Disease Modeling of Frontotemporal Dementia 3

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Frontotemporal dementia (FTD) is a common cause of early-onset dementia, with no currently available cure. Research is therefore crucial to elucidate disease mechanisms, to facilitate new therapeutic interventions. FTD3 is

a rare form of the disease, caused by a point mutation in the charged multivesicular body protein 2B (CHMP2B). This mutation has been observed to cause neuronal phenotypes, such as mitochondrial defects and abnormal endosomal-lysosomal fusion. However, the role of glial cells in FTD3 pathogenesis has until recently been rather unexplored. Neuroinflammation has been identified in most neurodegenerative disorders, with microglia potentially acting as proinflammatory drivers of the diseases. To assess the role of microglia in FTD3, we have derived induced pluripotent stem cells (iPSC) from a healthy individual, and by using CRISPR/Cas9 gene editing, established new knock-in cell lines, either heterozygous or homozygous for the CHMP2B mutation. These iPSC have been differentiated into both microglia and neurons, to observe how the mutation affect the two cell populations, and the microglia-neuron interaction. We have evaluated the proinflammatory profile of these microglia, performed metabolic tests, and investigated the microglial effect on neurons, to identify differences between our mutated cell lines and healthy control, and thus identify new disease phenotypes, specific for microglia. These findings have been further verified by RNA sequencing.

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T14-067D

Effects of influenza A virus infection on hippocampal neuron structure and function in aged wild-type mice

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Within the past hundred years, several pandemics have resulted from the emergence of novel influenza A virus (IAV) strains for which humans lacked immunity at the time when the virus occurred. Therefore, IAV strains pose a critical threat to public health. IAV infection typically manifests as a self-limiting respiratory syndrome, so far the nervous system involvement was only rarely acknowledged; however, an expanded spectrum of cerebral manifestations has been reported following IAV infection. These range from mild cognitive effects to seizures, depression and Parkinsonism, potentially increasing the risk for stroke, encephalitis, and neurodegenerative diseases. It is indicated that susceptibility to pulmonary infections such as influenza increases with age. Indeed, a progressive decline in the integrity of the immune system is one of the physiological changes during mammalian aging. The age-associated immune changes occur in every component of the immune system and result in increased production of proinflammatory cytokines and induction of a low-grade subclinical chronic proinflammatory status, also known as "inflammaging". We were therefore interested whether age is a risk factor for the development of a more pronounced and chronic immune response in the CNS of older individuals and whether this might subsequently pave the way for the development or exacerbation of neurodegenerative diseases. In this study, 15-month-old C57BL/6J mice were intranasally infected with non-neurotropic (H1N1 and H3N2) and neurotropic H7N7 IAV subtypes to investigate potential long-term effects on hippocampal structure and function in vulnerable individuals. Synapse loss detected at 30 dpi was associated with impaired spatial learning, reduced synaptic plasticity, and an increase in the population of activated phagocytic microglia along with elevated levels of

proinflammatory cytokines and chemokines including TNF-α, IFN-γ and CCL2. Moreover, evidence for synaptic stripping was detected in the hippocampus by increased phagocytic microglia engulfment of the postsynaptic compartment (IBA-1/ LAMP-1/Homer-1+ puncta). While neuroinflammation induced by the neurotropic H7N7 IAV showed the strongest effect, systemic infection with both non-neurotropic H1N1 and H3N2 subtypes also resulted in long-term impairments in synapse number and hippocampal function. Interestingly, at 120 dpi, a severe spatial learning deficit was still detectable in aged H7N7 IAV-infected mice which was not the case in young adult individuals. These results clearly demonstrate that IAV infection can lead to prolonged and more severe impacts on hippocampal function in older animals representing highly vulnerable individuals, reminiscent of the cognitive impact of "long COVID-19" symptoms.

T14-068D

Coexistance of different damage-associated myeloid populations in the hippocampus of Alzheimer's patients

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Parenchymal microglia are the brain-resident immune cells capable of responding to damage and disease. Though the role of microglial cells in the development/progression of AD is still unknown, a dysfunctional response has recently gained support since the identification of several genetic risk factors related to microglial function. In this sense, and clearly in contrast to that observed in amyloidogenic models, we have reported an attenuated microglial activation associated to amyloid plaques in the hippocampus of AD patients, including a prominent degenerative process of the microglial population in the dentate gyrus. On the other hand, it is also known that others myeloid components, apart from microglia, could also be involved in the neurodegenerative process. However, these different phenotypes and the implication of the diverse immune cells in the human pathology have not been determined yet. In this work, we analyzed the phenotypic profile displayed by damage-associated myeloid cells in the hippocampus of AD brains. For this purpose, immunohistochemistry and image analysis approaches have been carried out in p*ostmortem* frontal cortex from non-demented controls (Braak II) and AD cases (Braak V-VI). Damage-associated myeloid cells from Braak II and Braak VI individuals were clustered around amyloid plaques and expressed Iba1, TMEM119, CD68, Trem2 and $CD45^{high}$. A subset of these cells also expressed ferritin. However, and even though some Braak II individuals accumulated CD45-positive plaques, only AD patients exhibited parenchymal infiltration of CD163-positive cells (Iba1+/$CD45^{high}$/CD163+/MCR1-), along with a decrease of the resident microglial marker TMEM119. Moreover, a negative correlation was observed between CD163 and TMEM119 intensities in Braak VI patients, showing a functional cooperation among these different myeloid populations. The homeostatic and ramified microglial like cells of non-demented Braak II cases were characterized by Iba1, CX3CR1, P2ry12, TMEM119 and $CD45^{low}$ expression. Taken together, these findings suggest the existence of different populations of amyloid-associated myeloid cells in the hippocampus during disease progression. The differential contribution of these myeloid populations to the pathogenesis of the disease remains to be elucidated. The dynamic of the myeloid molecular phenotypes associated to AD pathology needs to be considered for better understand the disease complexity and, therefore, guarantee clinical success. Correcting dysregulated brain inflammatory responses might be a promising avenue to prevent/slow cognitive decline.

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T14-069D

Essential omega-3 fatty acids tune microglial phagocytosis of synaptic elements in the mouse developing brain

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Omega-3 fatty acids (n-3 PUFAs) are essential for the functional maturation of the brain. Westernization of dietary habits in both developed and developing countries is accompanied by a progressive reduction in dietary intake of n-3 PUFAs. Low maternal intake of n-3 PUFAs has been linked to neurodevelopmental diseases in Humans. However, the n-3 PUFAs deficiency-mediated mechanisms affecting the development of the central nervous system are poorly understood. Active microglial engulfment of synapses regulates brain development. Impaired synaptic pruning is associated with several neurodevelopmental disorders. We identify a molecular mechanism for detrimental effects of low maternal n-3 PUFA intake on hippocampal development in mice. Our results show that maternal dietary n-3 PUFA deficiency increases microglia-mediated phagocytosis of synaptic elements in the rodent developing hippocampus, partly through the activation of 12/15-lipoxygenase (LOX)/12-HETE signaling, altering neuronal morphology and affecting cognitive performance of the offspring. DHA, the main n-3 PUFA, highly accumulates in the brain over the perinatal period. Using genetically engineered mice allowing to increase n-3 PUFA in microglia (iFAT1:Cx3cre1creERT2), we investigate how early-life n-3 PUFAs impacts postnatal brain and establish a causal link between n-3 PUFA action on microglia and miswiring of the brain.

T14-070D

Involvement of neuroinflammation processes in nociceptive defects of *Fmr1* KO mice, model of Fragile X syndrome.

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Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability. FXS is due to large expansions of CGG repeat in *Fmr1* causing loss of FMRP (fragile X mental retardation protein) expression. Among the wide range of associated abnormalities, inadequate responses to environmental stimuli are described and could explain self-injurious behavior related to 60% of FXS patients. Although these sensorial disturbing features could contribute to the cognitive deficits and affect social interactions, the cellular and molecular processes involved are still poorly understood. To this purpose, our experiments using the *Fmr1* knockout mouse model (*Fmr1* KO) focused on tactile sensitivity in normal and inflammatory conditions.
In our experiments, *Fmr1* KO mice shown a hypersensitivity in chronic inflammatory condition. This inflammation

was induced by a unilateral paw administration of complete Freund's adjuvant (CFA) and tactile sensitivity was measured using Von Frey hairs. *Fmr1* KO mice displayed an excessive allodynia at least during 1 week after the injection as compared with their wild type littermates. When the Von Frey hairs are applied to the mice's paw, receptors such as nociceptors and mechanoreceptors are stimulated and the information is transmitted via nerve fibers to the ipsilateral posterior horn of the spinal cord. In the dorsal horn, these first fibers connect to spinothalamic tract to transmit information towards integrative centers of the cerebral cortex. Our hypothesis is that the hypersensitivity observed in *Fmr1* KO following CFA injection is due to a disturbance of neuroinflammatory state at the spinal cord level. Since, accumulating evidences suggest that neuroinflammation process is involved in the induction and maintenance of pain, we explored glial cells responses during the development of allodynia.
Using RT-qPCR assays, our data suggest an exacerbated reactivity of glial cells in *Fmr1* KO mice within the dorsal horn of the spinal cord but also in anterior cingulate cortex and in primary somatosensory areas. Since several evidences also suggested that astrocyte functions were disturbed in *Fmr1* KO mice, the astroglial responses to immune mediators/modulators were investigated using primary cells culture. Our preliminary data show for the first time a perturbation of immune responses of *Fmr1* KO astrocytes, in pathways that must be elucidated. Thus, defining immune function defects of *Fmr1* KO astrocytes could help to better understand their involvements to FXS physiopathology and to discover new therapeutic pathways.

T14-071E

Astrocytes and microglia are involved in myelin degeneration and repair by modulating the inflammatory environment

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Myelination of axons by mature oligodendrocytes (OLs) enables saltatory conduction of nerve impulses and provides trophic support to maintain axonal integrity. Loss of myelin is a hallmark of several neurodegenerative diseases. Microglia and astrocytes are glial cells involved in the demyelination, but also in myelin repair. Both cell types can modulate the molecular environment by releasing various pro- and anti-inflammatory factors, such as TNF-a, CNTF or IL-10, thereby affecting the survival of mature OLs as well as the proliferation and differentiation of oligodendrocyte precursor cell (OPC) at the lesion site. To study the dynamic interplay between these glial cells, we used a new focal and reversible lysolecithin (LPC)-induced demyelination model of the mouse spinal cord in combination with longitudinal two-photon imaging at day 0 (D0), D2, D4, D7, D14, and D21 after LPC application. Immediately after LPC, we observed recruitment and activation of microglia at the lesion site. In contrast, the hypertrophic activation of astrocytes required four days before it could be detected. The *in vivo* image recordings were complemented by a qRT-PCR analysis of FACS sorted $Cd11c^{+}$microglia (D4, D7 and D14) demonstrating an upregulation of the pro-inflammatory genes TNF-a and IL-6 till D7 after LPC application. During this pro-inflammatory phase, we observed a loss of mature OLs by reduced $CC1^{+}$ immunostaining. Subsequently, from D7 to D14, anti-inflammatory genes such as arginase 1 were found to be up-regulated. We hypothesize that microglia determine the inflammatory status of the CNS directly after an LPC injury.

T14-073E

Anxiety and mild microglial activation in the amygdala two weeks after NA-induced neuroinflammation

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A single injection of neuraminidase (NA) within the cerebral ventricles (ICV) triggers an acute neuroinflammation, which is largely solved in two weeks. Neurological complications or behavioral alterations have been associated to neuroinflammation. While some of these symptoms decline with time along with inflammation, the possibility of long-term sequelae should be considered. Thus, we aimed to explore if NA-induced neuroinflammation provokes behavioral or neurological disturbances at medium (2 weeks) and long (10 weeks) term. Rats were ICV injected with NA or saline. A battery of neurological tests and a behavioral assessment (open field test) were performed 2 and 10 weeks post-ICV. Also, the inflammatory status was evaluated by immunohistochemistry and qPCR. First, neurological alterations of the sensorimotor reflexes were not found, suggesting that NA does not cause disturbances in major brain functions. While the open field test revealed normal locomotor capacity in the animals injected with NA, however the evaluation of specific behaviors (*rearing* and *rearing with support*) pointed out an increased anxiety state 2 weeks after NA administration, but not at long term (10 weeks). These results were confirmed by analyzing all the behavioral parameters measured in the open field test by means of a principal components analysis. Regarding signs of neuroinflammation, an overexpression of some genes related to inflammation (the receptor TLR4 and the alarmin HMGB1) was found in the hypothalamus of NA treated rats at 2 weeks post-ICV, but not at 10 weeks. A histological study of brain areas related to emotions (amygdala) and stress response (hypothalamic PVN) revealed no significant differences in the number of microglia or astrocytes. Nevertheless, the morphological analysis of microglial cells (a quite sensitive tool to evaluate microglial activation) demonstrated that, in the amygdala of NA injected rats, microglia presented a morphology consistent with a slightly activated state (decreased *cell area*, *cell perimeter*, *fractal dimension* and r*oughness*; increased *cell circularity* and *lacunarity*). Such morphological change, which was evident 2 weeks after NA injection, was virtually reverted 10 weeks post-ICV. A similar study performed in PVN microglia yielded very subtle morphological changes (mostly not statistically significant). These results point out that NA injected ICV may cause anxiety in the medium term (while not affecting other functions like sensorimotor functions or the locomotor capacity), a behavioral alteration that is transient and that concurs with a mild inflammation, evidenced by the overexpression of certain genes and, more notably, by the morphological bias of microglial cells located in the amygdala towards an activated profile.

T14-074E

NEMO differentially affects astrocytes and microglia by NF-κB-dependent and –independent functions following brain injury

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Neurodegenerative diseases that are characterized by a strong inflammatory component are invariably accompanied by a prominent reactivity of microglia and astrocytes, as following traumatic and ischemic brain injury. Recent studies have shown that both, reactive microglia and astrocytes are characterized by distinct populations of multiple cellular states mostly defined by their inflammatory outcome. Furthermore, it has been described that NF-κB signalling is strongly associated with neuroinflammation, glial reactivity as well as neurodegeneration. However, the exact contribution of Nemo-NF-κB-dependent mechanisms regulating astrocyte and microglia reactivity as well as tissue resolution after brain injury remains poorly understood. Here, we could show temporal differences in glial cell activation and NF-κB dependent gene expression with label-free proteomic analysis of FACS sorted microglia and astrocytes following controlled cortical stab-wound (SW) injury *in vivo*. Monitoring NF-κB nuclear translocation with the use of a GFP-p65 (NF-κB subunit) knock-in mouse confirmed that NF-κB activation was mostly restricted to microglia and astrocytes with markedly distinct temporal dynamics after brain injury. In particular, microglia displayed p65 nuclear translocation as early as 1h after injury, whereas NF-κB-mediated astrocyte activation appeared delayed, suggesting that astroglial cells mostly contribute to later stages of the inflammatory response. To reveal the selective contribution of this temporally shifted activation of NF-κB, we generated two mouse lines to conditionally ablate the master regulator of NF-κB activation (NEMO) either in microglia or astrocytes during injury. Time course experiments and 2photon laser scanning microscopy (2PLSM) chronic *in vivo* imaging demonstrated differential changes in proliferation and survival in each of the glial cell types upon deletion of NEMO. Interestingly, microglia specific p65 knock-out did not show microglia loss upon brain injury, suggesting that NEMO prevents microglia death following injury by NF-κB-independent functions different to astrocytes.

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T14-075E

Leriglitazone decreases microglia activation and promotes protective phenotypes in several neuroinflammatory models of disease

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Microglia when activated produce pro-inflammatory cytokines and become highly phagocytic which can exacerbate neuronal loss in many neurological conditions where inflammation is involved. However, microglia display a huge plasticity in response to injury and promote tissue repair and resolution of inflammation. Thus, decreasing microglia activation and promoting beneficial phenotypes could be an effective strategy to halt brain damage. PPAR gamma

agonists have shown promising results at mediating anti-inflammatory responses and polarizing microglia towards pro-regenerative phenotypes, but their insufficient brain penetration has hampered their clinical development. We show in this study that leriglitazone (also known as MIN-102), a selective PPAR gamma agonist with improved profile for CNS diseases, decreases microglia activation in several neuroinflammatory in vivo and in vitro models of X-linked adrenoleukodystrophy neurodegenerative disease, LPS-induced inflammation, and glutamate induced excitotoxicity models. Moreover, leriglitazone improved the phagocytosis of myelin debris and decreased the phagocytosis of beads under inflammatory conditions, thus promoting beneficial roles of microglia in demyelinating diseases and neuroinflammation. These findings support the use of leriglitazone for the treatment of neuroinflammatory diseases. Leriglitazone has been recently tested in a phase 2/3 clinical trial for the treatment of neurodegeneration in Adrenomyeloneuropathy and a phase 2 trial for Fredreich's Ataxia and is currently being tested in Cerebral Adrenoleukodystrophy.

T14-076E

Exploiting astrocyte-specific neuroprotective and neuroregenerative mechanisms to stop progressive MS

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Multiple sclerosis (MS), being the the most frequent chronic inflammatory disease of the central nervous system, is marked by a highly heterogeneous disease course. Patients can undergo a mild type without suffering from MS related disability to a severe, ultimately heavily disabling form of the disease. The cause for this diverging disease progression has not been elucidated yet. We hypothesize, that CNS endogenous cells, such as astrocytes, contribute to neuroprotection and –regeneration and are decisive for the clinically diverse disease courses. The underlying mechanisms and the extent of neuroprotection and –regeneration in particular by CNS endogenous cells are largely unknown. In our study, we focused on the role of astrocytes and their potential to protect neurons from inflammation-mediated damage.

We generated induced pluripotent stem cell (iPSC) lines from 6 individual MS patients (3 patients with a benign disease course, i.e. EDSS <3 and 3 patients with a disabling disease course, i.e. EDSS >6). These iPSCs were differentiated into astrocytes to establish a co-culture model of patient derived astrocytes together with a healthy control of an inducible neuronal cell line (neurogenin2, NGN2) to study inflammation related neuronal damage and protection in MS. To simulate (pro)-inflammatory stress on neurons, we analyzed immunofluorescence staining against SMI-32, an antibody recognizing an epitope of the non-phosphorylated Neurofilament H (NF-H) variant and a surrogate marker for neuronal damage. Our results showed that neurons in co-cultures with astrocytes derived from benign patients were protected against damage-inducing pro-inflammatory cytokines TNFα and IL17. Contrastingly, co-cultures of neurons with astrocytes from disabling patients were not protected against these cytokines, shown by significantly increased proportions of SMI-32 positive neurons. Monocultures of neurons derived from the different patient subsets treated with cytokines did not show clear differences between the patient subsets.

Based on our results we conclude that astrocytes from benign MS patients effectively contribute to prevent signs of neurodegeneration in a novel preclinical model for immune mediated neurodegeneration. To further exploit this

model we want to investigate the transcriptome of these co-cultures to find mechanism of astrocyte driven neuroprotection. We think that this model will contribute to explain the rare phenomenon of benign MS and to find potential drugable targets that support neuroregeneration and -protection in MS patients.

T14-077E

Role of CB2 receptor in the 5x FAD mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid-beta peptide (Aβ) in neuritic plaques within the brain leading to a strong inflammatory response. Previously, our group observed an increase in the expression of CB_2 receptors which were shown to be selectively expressed by microglial cells located in the vicinity of amyloid-enriched neuritic plaques. In addition, CB_2 agonists have been shown to improve cognitive impairment as well as neuroinflammation and microgliosis animal models of this desease.

We isolated adult microglia cells from the brains of several 5xFAD-related mice models: i) mice in which CB_2 expression is linked to GFP expression (5xFAD/$CB_2^{EGFP;f/f}$); ii) mice in which CB_2 receptor has been constitutively deleted (5xFAD/$CB_2^{-/-}$); and, iii) mice in which CB_2 expression is deleted in a time-dependent manner and specifically in microglial cells (5xFAD/$CB_2^{EGFP;f/f}$/$Cx3cr1^{tm2.1(cre/ERT2)Jung}$). We analyzed the phagocytic activity of these cells, the expression of cytokines (IL1b, TNFa, IL6), markers of the M1 and M2 polarization and molecules involved in the inflammatory response (pNFkB, COX, caspase-1). Deletion of CB_2 receptor caused an impairment in the phagocytic activity of microglial cells and also induced changes in the cytokine expression profile.

We can conclude that CB_2 receptors exert a modulatory effect in neuroinflammatory responses of microglia and changes in their ability to phagocytose amyloid peptides. These data suggest that CB_2 receptor expressed in microglial cells may play a significative role in the neuroinflammation in the context of AD.

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T14-078E

Microglia selective depletion delays EAE onset

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Microglia are the resident immune cells of the central nervous system (CNS), and they are involved in the development of diverse neurodegenerative processes. In multiple sclerosis and the experimental autoimmune encephalitis (EAE) mouse model, two pools of morphologically indistinguishable phagocytic cells, parenchymal microglia and infiltrated macrophages, contribute to demyelination and axonal damage. However, spontaneous yet transient myelin repair can occur during the course of the disease model and the innate myeloid response is a key component of this regenerative process. Nevertheless, the specific roles of the microglial population versus macrophages are unknown. Using the colony stimulating factor 1 receptor (CSF1R) inhibitor, PLX5622, to selectively deplete microglia cell population, and the CCR2-RFP/fms-EGFP mice to distinguish peripheral macrophages and microglia, we addressed the specific role of microglia in EAE. Microglia selective depletion led to a massive infiltration of peripheral CCR2-RFP^{+} macrophages into the white matter demyelinating lesions but also into the whole spinal cord parenchyma, including grey matter. Despite this massive infiltration of macrophages, PLX5622-treated mice showed no changes in clinical signs at chronic EAE, suggesting that microglia are not relevant for better outcome or more efficient remyelination. However, microglia depletion induced a significant delay in EAE onset. Immune priming in the periphery, including lymph nodes, spleen and blood, as well as the recruitment of immune cells at the meninges, was not altered. However, microglia elimination reduced the expression of the CD80 co-stimulatory molecule in dendritic and myeloid cells and reduced T cell reactivation and proliferation in the spinal cord parenchyma. Altogether, this data points to a specific role of microglia in antigen presentation and T cell reactivation at initial stages of the EAE model.

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T14-079E

Modelling epilepsy seizures in vitro: A kainic acid-induced seizure model for studying the effect of IL-6 on the characteristics and functionality of hPSC-derived cortical neurons

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Background and aims: Epilepsy is a common neurological disorder characterized by repetitive, unpredictable epileptic seizures. Neuroinflammation and abnormal inflammatory cytokine levels have been associated with epilepsy. Animal and clinical studies have shown that the levels of interleukin-6 (IL-6) cytokine are elevated after seizures. However, the knowledge of the roles of IL-6 in seizures and its functions on human neurons is minimal. Human pluripotent stem cell (hPSC) -derived neurons are promising toolfor in vitro modelling of epilepsy or seizure-like activity. In this study, we developed in vitro kainic acid (KA)-induced seizure model and studied the effect of IL-6 on the characteristics and functionality of hPSC-derived cortical neurons.

Methods: Gene and protein expression levels of IL-6 receptors, IL-6R and glycoprotein 130 (gp130), on hPSC-derived cortical neurons were studied with quantitative PCR (qPCR) and immunocytochemistry (ICC), respectively. Functionally maturated cortical neuronal networks were used to model KA-induced seizure-like activity. Microelectrode arrays (MEAs) were used in functional measurements. The effects of IL-6 and hyper-IL-6 (H-IL-6) fusion protein treatment on the cortical neuronal network activity were studied. Live/Dead assay was used to study the effects of KA and cytokine treatments on the cell viability.
Results: Functionally mature hPSC-derived cortical neuronal networks responded to KA treatment with an excessive bursting phenotype, but KA treatment was not cytotoxic for the cells. Neuronal cultures expressed both IL-6 receptors, IL-6R and gp130, at the gene and protein level. Treatments with IL-6 or H-IL-6 were not cytotoxic to the cells, but increased the gene expression levels of IL-6R. Cytokine treatments did not alter the activity of cortical networks.
Conclusion: This study demonstrated that combining the hPSC-derived cortical neurons, MEAs and KA as a seizure-inducing agent are promising tools for modelling epilepsy in vitro. Moreover, expression levels of IL-6R were increased after IL-6 and H-IL-6 treatments suggesting that human neurons can respond to IL-6 through classical and trans-signaling pathways. However, acute treatment with IL-6 and H-IL-6 did not modulate functional activity, suggesting that IL-6 does not affect the induction or modulation of newly induced seizures in healthy cultures. Overall, we propose this model as a useful tool for studying seizure-like activity in human neuronal networks in vitro.

T14-080E

MT5-MMP is a modulator of inflammation: a study in murine and human neural cells related to Alzheimer's disease

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Neuroinflammation is a double edge sword with either protective or detrimental consequences. We have previously discovered the involvement of the matrix metalloproteinase MT5-MMP in the pathophysiological processes linked to Alzheimer's disease (AD). MT5-MMP deficiency in the 5xFAD mouse model of AD strongly reduces the accumulation of Abeta and C99 and tunes down neuroinflammation, all associated with the prevention of deficits in LTP and learning. However, the impact of MT5-MMP on neuroinflammation at pre-symptomatic stages of the pathology and its possible interplay with amyloidogenesis and synaptic activity is still elusive. To address this question, we investigated the role of MT5-MMP in primary mouse neuron/astrocyte cultures at 21 DIV and iPS-derived astrocytes from non-AD and AD patients using molecular and cell biology, biochemistry, immunocytochemistry and electrophysiology.
We showed that in primary neural cell cultures from 5xFAD mice, the absence of MT5-MMP (knock-out) reduced the inflammatory response to IL-1beta at early stages of development as well as APP metabolism and the levels of APP C-terminal fragment (C83). Moreover, in 5xFAD cells MT5-MMP deficiency prevented the loss of dendritic spines and neuronal hyperexcitability. Interestingly, MT5-MMP deficiency also negatively impacted synaptic integrity in the non-AD background. Leveraging on CRISPR/Cas9 technology to suppress MT5-MMP in iPS-derived

astrocytes, we showed that the lack of MT5-MMP promoted the response to a cocktail of inflammatory mediators (IL-1beta, TNF-alfa and MCP-1) in cells from non demented individuals (ND), whereas it attenuated such response in cells from AD patients (with duplicated App gene). We also revealed for the first time that MT5-MMP influences APP metabolism in astrocytes. We showed a marked tendency to decrease APP levels in isogenic MT5 KO clones in the ND group, while the opposite effect was observed in APPDp astrocytes, where full-length APP levels were increased. Moreover, the lack of MT5- MMP decreased the levels of alfa/beta-CTFs in ND astrocytes, while it increased them in APPDp cells.

These results in murine and human cells consolidate the potential contribution of MT5-MMP in AD and support our previous results (Baranger et al., 2017; Baranger et al., 2016).

Overall, our results highlight MT5-MMP as a pivotal proteinase in the control of neuroinflammation, amyloid metabolism and synaptic plasticity, and paves the way for the modulation of MT5-MMP as promising therapeutic target at pre-symptomatic phases of AD.

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T14-081E

Reduced microglial responses to persistent joint inflammation in a model of Alzheimer’s disease.

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Accumulating evidence implicates significant alteration in pain perception in patients with Alzheimer’s disease (AD). Systemic inflammation in conditions associated with chronic pain, such as Rheumatoid Arthritis (RA), correlates with increased risk of developing dementia. These observations highlight the necessity to examine the mechanisms underlying altered chronic pain in AD. Here, we focus on microglia, as they respond to systemic inflammation, neurodegeneration as well as increased nociceptive pathway activity. Our aim is to evaluate whether alterations in microglial responses in pain-related areas of the spinal cord and the brain contribute to the inflammatory pain mechanisms in a mouse model of Alzheimer’s disease.

As a model of persistent inflammatory pain, we used the K/BxN serum transfer model of inflammatory arthritis in the TASTPM transgenic mouse model of AD. Upon passive immunization with the K/BxN serum, mice developed severe joint swelling at their paws, peaking at day 5 after serum transfer, which later spontaneously resolved by day 30. The K/BxN serum transfer was also associated with persistent hind paw mechanical hypersensitivity (allodynia), both during and after the resolution of joint swelling in WT. We observed that the TASTPM mice

developed significant mechanical hypersensitivity at Day 5 after serum transfer. However, allodynia was significantly attenuated from day 17 to day 30 compared to WT treated with K/BxN serum.
Following the behavioural studies, we have examined microglial activation at 5 and 30 days after K/BxN or control serum transfer, as assessed by staining for IBA1$^+$/p-p38$^+$ cells in the dorsal horn of the spinal cord and microgliosis (IBA1$^+$ cells) the ventral posterior (VP) thalamic nuclei. In WT dorsal horns at both day 5 and day 30, the number of activated microglia was higher in K/BxN than control serum groups. However, the dorsal horn of TASTPM treated with K/BxN displayed a higher number of activated microglia than TASTPM treated with control serum at day 5, but not at day 30. Microgliosis was also observed at the VP nuclei of WT at day 30, but not day 5 after K/BxN serum transfer. In TASTPM treated with K/BxN, pronounced microgliosis was observed in the thalamus at both day 5 and day 30 after serum transfer. Moreover, K/BxN treatment in TASTPM was associated with exacerbation of the amyloid pathology both at day 5 and day 30, as increased number of amyloid plaques was detected in the thalamus.
This data suggests that i) increased microglial responses in pain-related areas are associated with mechanical hypersensitivity in inflammatory arthritis and ii) diminished spinal microglia responses might be involved in the mechanisms underlying the reduced nociception in persistent joint inflammation in the TASTPM.

T14-082E

Reactive phenotype of astrocytes pre-exposed to CSF from multiple sclerosis patients with inflammatory phenotype.

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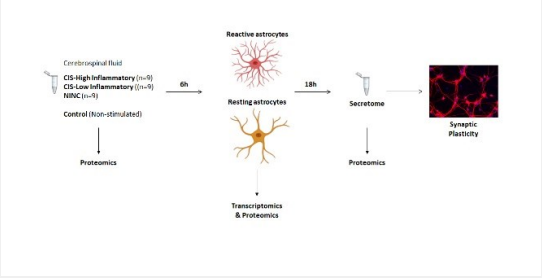
Several lines of evidence point to astrocytes as important contributors to the neurodegenerative component observed in MS. Here, we aimed to study whether a MS-specific highly inflammatory microenvironment induces a more aggressive reactive phenotype on astrocytes that leads to neuronal damage. First, we optimized the stimulation and secretome collection conditions (6+18h vs. 12+12h) by challenging purified primary cultures of mouse astrocytes with two different cerebrospinal fluid (CSF) pools from patients with a high inflammatory radiological phenotype at the first demyelinating event or clinically isolated syndrome (CIS-High, n=2 patients/pool). Astrocytes exposed to CIS-High in 6+18h condition became more reactive and displayed a significant dendrite length retraction (p=0.007 vs. Control). Stimulation of 6h and 18h of collection times were chosen for subsequent experiments. Next, astrocyte cultures were stimulated with medium (Control) or CSF from: (i) Patients with high (CIS-High, n=9) and (ii) low (CIS-Low, n=9) inflammatory radiological phenotypes; and (iii) non-inflammatory

neurological disease controls (NINC, n=9). We generated 3 CSF pools per group (n=3 patients/pool). Secretome, RNA and intracellular protein samples from astrocytes were collected to perform: (i) a molecular characterization of astrocyte reactive phenotype by transcriptomics and intracellular and secretome proteomics; and (ii) functional studies based on secretome effect on synaptic plasticity. The gene expression study performed using microarrays showed significantly differentially expressed genes in CIS-High-activated astrocytes compared to non-inflammatory conditions (87 vs. Control, 23 vs. NINC, 7 vs. CIS-Low). Intracellular proteomics based on mass spectrometry analysis also exhibited significant differential expression patterns (58 vs. Control, 51 vs. NINC, 49 vs. CIS-Low). Moreover, we identified 12 pro-inflammatory molecules significantly increased in CIS-High (vs. CIS-Low). Therefore, astrocytes exposed to a highly inflammatory microenvironment acquire a specific reactive pro-inflammatory signature.Then, we treated cortical neurons (18DIV) with secretomes for 72h. We observed a significant reduction of PSD-95 and active synapsis density in CIS-activated astrocyte secretomes compared to non-inflammatory controls (PSD-95: $p<0.001$ vs. Control, $p=0.04$ vs. NINC; synapsis: $p<0.001$ vs. Control). Particularly, CIS-High had a more prominent effect on dendrite length retraction when comparing to all conditions ($p<0.0001$). Finally, significant higher levels of SerpinE1 were found in CIS-High secretomes ($p=0.01$ vs. CIS-Low) which might be the mediator of the non-cell autonomous effect of CIS-High pre-exposed astrocytes on synaptic plasticity.



Graphical abstract

T14-083E

Complement cascade-mediated synapse elimination in the developing hippocampus is synapse- and region-specific

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The complement cascade is an innate immune pathway that is a key regulator of synaptic pruning by microglia during brain development. In multiple disorders including Alzheimer's disease (AD) and schizophrenia, there is substantial activation of the complement cascade in the hippocampus which leads to synapse loss and cognitive impairment. It is widely thought that this loss of synapses is due to the pathological reactivation of the complement-dependent developmental pruning program. Understanding the fundamental mechanisms underlying complement-

dependent pruning in development will provide insight into how this process becomes aberrantly active in disease.

The hippocampus is a primary affected brain region in AD and schizophrenia, however, it is unknown which synapses in the hippocampus undergo complement-dependent pruning in normal development. Using immunohistochemistry in the second postnatal week, we have found that in C3 KO mice there is increased VGLUT2, but not VGLUT1, levels and co-localization with the post-synaptic marker Homer1 in the CA1 stratum lacunosum moleculare (SLM) and dentate gyrus molecular layer. Further, there was a corresponding decrease of microglia phagocytosis markers in these regions. Finally, in the CA1 stratum radiatum (SR), which contains only VGLUT1 synapses, there was no change in VGLUT1 levels, co-localization with Homer1 or microglia phagocytosis markers. This study provides new insight into how the complement cascade controls synapse number in the developing hippocampus. We have found that synapse elimination through complement is not a ubiquitous feature of all synapses, but is instead restricted to VGLUT2-containing synapses in the CA1 SLM and dentate gyrus. These findings will be crucial for understanding how this process of synapse-selective elimination via the complement cascade goes awry in neurodegenerative diseases of the hippocampus such as AD.

T14-084E

Intracellular trafficking modulation by the cholesterol-dependent cytolysin pneumolysin from *S. pneumoniae* is critical for the initiation of neuroinflammatory response

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During *Streptococcus pneumoniae* (pneumococcal) meningitis and other bacterial infections of the brain, bacteria multiply in the cerebrospinal fluid and subsequently undergo spontaneous lysis once reaching a critical density. In this process, multiple pathogenic factors such as the cholesterol-dependent cytolysin pneumolysin are released. Earlier works indicate that pneumolysin is critical for the neuroinflammatory response through the activation of Toll-like receptors. Here, we outline the importance of the enhanced endocytosis by the cytolysin as a major factor that facilitates the delivery of neuroinflammatory ligands to their cytosolic receptors. Inhibition of the toxin-enhanced endocytosis by the dynamin inhibitors Dynasore, Dyngo4a, and MitMab nearly completely inhibited the release of the inflammatory cytokines TNF-α and IL-6. Further analysis of the enhanced endocytosis showed that it was potassium-dependent and calcium-independent. Pneumolysin-GFP was also internalized, but in a dynamin-independent manner, suggesting that the toxin does not share the internalization pathways involved in the proinflammatory response, but can enhance them. The fluorescent toxin co-localized with flotillin-positive endosomes. Finally, pharmacological inhibition of the endocytosis in C57Bl/6 mice with experimental meningitis diminished the neuroinflammatory response, confirming the relevance of the mechanism in vivo. Our work reveals a novel control mechanism of the neuroinflammation in pneumococcal brain infection through the modulation of the endocytosis by a key bacterial virulence factor - the protein toxin pneumolysin.

T15 | Neurovascular interactions

T15-001A

Increased expression of chemokine receptor CXCR4 in astrocyte endfeet at the neuro-vascular unit from mesial temporal lobe epilepsy patients with hippocampus sclerosis.

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Mesial temporal lobe epilepsy (MTLE) is the most common form of refractory epilepsy, characterized by spontaneous recurrent seizures. Functional impairment of the blood-brain barrier (BBB) has been attributed to contribute to the formation and/or progression of the disease. However, a detailed knowledge of the molecular changes at the BBB and the neurovascular unit (NVU) is currently missing. This study aims to characterize BBB properties affected by epilepsy or affecting the progress of epilepsy. To this end, human microvessel fragments, resembling the NVU, of morphologically unaffected cortex and epileptic hippocampus tissue of MTLE patients were compared. RNA-Seq revealed significantly dysregulated genes in epileptic hippocampus, being related with cadherin signaling, negative regulation of canonical Wnt signaling, vascular permeability, and inflammatory response. Among these genes, CXCR4 was found to be overexpressed in epileptic and sclerotic hippocampus. The overexpression of the gene has been validated by real-time polymerase chain reaction (qRT-PCR). Immunohistochemistry on paraffin-embedded sections revealed increased expression of CXCR4 specifically in the sclerotic hippocampus. Immunofluorescence double-staining on cryosections showed that the CXCR4 signal colocalizes with astrocyte endfeet at the BBB. Our results demonstrate the pathological change in the astrocyte endfeet at the NVU in sclerosis. To examine the role of CXCR4 expression on the BBB and to clarify whether the high expression levels of CXCR4 in the sclerotic hippocampus have a direct influence on the tightness of the BBB, overexpression and silencing of the gene will be performed in astrocytes. To analyze the effect of the modified expression levels of the CXCR4 in astrocytes on the BBB properties, these astrocytes will be co-cultured with brain microvessel endothelial cells (BMECs) and transendothelial electrical resistance (TEER) measurements will be performed in vitro on this co-culture set-up. Preliminary TEER experiments revealed that treatment of astrocytes with CXCR4 antagonist AMD3100 may lead to better barrier formation of BMECs, suggesting its protective role on the BBB.

T15-002A

Expression of the MLC1/GlialCAM complex in perivascular astrocyte endfeet defines a temporal window for the postnatal gliovascular unit maturation

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Astrocytes, the most abundant glial cells in the brain, are voluminous, highly ramified, project long processes to neurons and brain vessels, and dynamically regulate distal synaptic and vascular functions. At the vascular interface, perivascular astrocytic processes (PvAPs, often called endfeet) form a continuous layer around the brain vessels. Via the PvAPs, astrocytes control several brain vascular functions, including the integrity of the blood-brain barrier, the homeostasis between the brain and the immune system, the transfer of metabolites, and the regulation of cerebral blood flow. Most of the astrocytes' perivascular functions rely on a specific molecular repertoire that is enriched in PvAPs (Cohen-Salmon et al., 2021). How the gliovascular interface develops has not been fully investigated.

We recently characterized the postnatal expression of MLC1 and GlialCAM, two transmembrane proteins forming a complex enriched at the junctions between mature astrocyte perivascular endfeet. We showed an enrichment of the two proteins in astrocyte endfeet between postnatal days 10 and 15. This event correlated with the increased expression of Claudin-5 and P-gP, two endothelial-specific BBB proteins suggesting that astrocyte perivascular endfeet and BBB maturate in concert between P10 and P15, and that the formation of MLC1/GlialCAM complex might be a key event in the gliovascular unit maturation (Gilbert et al., 2019).

The Megalencephalic Leucoencephalopathy with subcortical Cysts (MLC) is a rare genetic disease linked to MLC1 and/or GlialCAM dysfunction and characterized by the development of brain anomalies from the first year of life in humans (Estevez et al., 2018). Following our characterization of the MLC1/GlialCAM complex, we propose that MLC might be related to an abnormal maturation of the gliovascular unit.

Acknowledgement

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T15-003A

Role of Hippocampal Glio-Vascular Interface in Sex- Dependent Outcome following Neonatal Inflammation

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Background: The neuro-gliovascular unit is crucial for providing a balanced CNS environment and consequently well-functioning neurons. Activation of the immune system during the developmental period is believed to affect the gliovascular unit and trigger neurodevelopmental disorders such as autism. Accordingly, we investigated alterations of the hippocampal gliovascular unit following early life inflammation as a crucial component underlying higher susceptibility of the male brain to autism.

Methods: Male and female C57BL/6J pups received a single injection of lipopolysaccharide (LPS) (1mg/kg) or saline on postnatal day 5 (P5) (n=24, ♂=12, ♀=12). Forty days after LPS/saline injection, the mice were tested for autistic-like behaviors (sociability and repetitive behavior). Thereafter, the brains were collected and immunostained for GFAP, Iba1 and CD31 to identify components of the gliovascular unit (astrocytes, microglia and microvessels) in the molecular layer of dentate gyrus (MDG) in the dorsal and ventral parts of hippocampus. Cell soma size and vessel density were analyzed using unbiased stereological techniques.

Results: Significant abnormal repetitive behavior was observed in LPS-injected male and female mice compared to the saline groups with more prominent alteration in male mice. Sociability was significantly affected only in LPS-injected male mice. Significant differences in morphology of the gliovascular unit were observed between LPS-injected and saline-injected male mice represented by smaller microglia soma size in ventral MDG and larger astrocytes soma size in the ventral and dorsal MDG. Compared to saline-injected males, the LPS-injected males demonstrated shorter microvessels in the ventral MDG. No significant differences in morphology of the gliovascular unit were observed among female mice.

Conclusion: Neonatal inflammation may induce susceptibility to autism through modification of hippocampal gliovascular interface in a sex-dependent manner.

T15-004A

Extracellular vesicles from astrocytes of 3TG-AD mice fail to suppress tert-Butyl hydroperoxide-induced mitochondrial oxidative stress in brain endothelial cells

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Mitochondrial dysfunction and increased oxidative stress in endothelial cells of brain vasculature can impair blood-brain barrier (BBB) function and contribute to the development of Alzheimer's disease (AD). Astroglial dysfunction, occurring in the early stages of the AD may be similarly critical for BBB damage. In the present study, we compared effects of extracellular vesicles (EVs) derived from immortalized astrocytes prepared from hippocampi of triple transgenic AD model mouse (3xTG-AD) and wild-type controls (3Tg-iAstro and WT-iAstro, respectively) on the tert-Butyl hydroperoxide (tBHP)-induced mitochondrial oxidative stress in immortalized human brain endothelial cells (hCMEC/D3s). We also compared proteomic profiles of EVs from WT-iAstro and 3Tg-iAstro related to oxidative stress.

EVs were purified by differential ultracentrifugation from WT-iAstro and 3Tg-iAstro lines cultivated in the EV-depleted medium. Oxidative stress in hCMEC/D3s was induced with tBHP (120 μM) while production of mitochondrial reactive oxygen species (ROS) was detected by confocal microscopy using MitoSOX Red probe. Shotgun proteomics and subsequent gene ontology (GO) analysis were used to compare EVs derived from WT-iAstro and 3Tg-iAstro.

We found that 6 hours incubation with tBHP increased mitochondrial ROS production in hCMEC/D3s by 22.40 ± 5.54 % (n = 3) compared to control. Pretreatment with EVs from WT-iAstro for 1 hour suppressed tBHP-induced mitochondrial ROS production by 11.62 ± 6.05 % (n = 3) compared to tBHP-treated cells, whereas pretreatment with 3Tg-iAstro-derived EVs had no significant effect.

Analysis of GO terms associated with oxidative stress identified 38 differentially expressed proteins. Majority of these proteins (33 of 38) were downregulated (by > than 1.5 fold) in EVs derived from 3Tg-iAstro.

In conclusion, we demonstrate that EVs from 3Tg-iAstro, in contrast to EVs derived from WT-iAstro, can not suppress tBHP-induced mitochondrial oxidative stress in hCMEC/D3s. These effects may, at least partially, depend on the downregulation of antioxidant proteins in EVs from 3Tg-iAstro. We suggest that EVs secreted by dysfunctional AD astroglia lost their ability to protect brain capillary endothelial cells against oxidative stress.

T15-005A

Cerebral Small Vessel Disease novel rat model: endothelial cell dysfunction affects oligodendroglia maturation

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Cerebral small vessel disease (cSVD) is the leading cause of vascular dementia and triples patients' risk of stroke. The crosstalk between endothelial cells of the blood-brain barrier and oligodendroglia of the white matter is an emerging field in the context of neurodegenerative disorders and has potential to help understand how the vessel changes in cSVD lead to cognitive changes in patients, and may lead to potential new targets for therapies.

An existing rat model of cSVD indicates that the underlying cause is not simply hypertension but an inherent dysfunction in endothelial cells of the blood-brain barrier, which causes a maturation block on the oligodendrocytes

of the white matter (Rajani et al., 2018). Previously we showed that this rat model has a homozygous deletion mutation of the flippase ATP11B, which is sufficient to cause endothelial dysfunction, and that single nucleotide polymorphisms in ATP11B are associated with humans with sporadic cSVD.

To better elucidate the effects of endothelial dysfunction in this disease, this work characterises a novel ATP11B knock-out (ATP11BKO) transgenic rat to examine how well it reflects cSVD pathology. The ATP11BKO rat will provide a platform to study endothelial dysfunction in the absence of hypertension, investigate impact of glial cells and offer a new model of cSVD to trial new approaches to tackling this disease.

The ATP11BKO animal is shown to be normotensive but to exhibit key features of cSVD. Endothelial cell dysfunction is demonstrated with key markers including loss of tight junction marker CLDN5, increased levels of ICAM-1 and reduced vessel lumen size. Next, effects on oligodendroglia are shown by reduced maturation and changes in vitro to myelination as a direct result of this dysfunction. Furthermore, the scope for the ATP11BKO animal as a model for cSVD is suggested by broader white matter changes in vivo. In particular, the difference between young and older animals of this genotype highlights a potential vulnerability in oligodendroglia that may suggest a mechanism for cSVD.

Together, this data lays out characterisation of key features of a novel transgenic animal that offers a potentially useful platform for investigating normotensive cSVD and endothelial cell dysfunction.

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T15-006B

Role of astrocytes in the dopaminergic modulation of the vascular tone in the spinal cord

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Astrocytes are key players in the adjustment of blood flow to local neuronal activity and in the regulation of the vascular tone in the central nervous system (Attwell et al., 2010). Astrocytes are ideally positioned at the interface between local neuronal circuits and blood vessels. They have microprocesses surrounding synapses and endfeet wrapped around parenchymal blood vessels. The activation of astrocytes by neurotransmitters induces an increase in intracellular calcium which can in turn lead to the release of vasoactive compounds. It was recently found that dopamine can activate astrocytes in the nucleus accumbens (Corkrum et al., 2020). Because dopamine is also released in the spinal cord by descending neurons originating in the A11 area in the hypothalamus (Björklund et al., 1979; Sharples et al., 2014), we decided to investigate the effect of dopamine on spinal astrocytes. We performed two-photon calcium imaging of astrocytes in isolated spinal cord preparations and slices from neonatal mice. We found that astrocyte endfeet responded to dopamine application by intracellular calcium increases. Responses to dopamine persisted in the presence of a cocktail of neurotransmitter receptor antagonists - blocking AMPA, NMDA,

glycine, $GABA_A$, $GABA_B$ and metabotropic glutamate receptors - and of the sodium channel blocker tetrodotoxin. This observation suggests that the effect of dopamine was not mediated by neuronal activation. Responses to dopamine were blocked by addition of the selective antagonist of dopamine receptors type 1, SCH 23390. Dopamine induced a constriction of arterioles which was also blocked by SCH 23390. Preliminary analyses suggest that calcium increases in astrocytic endfeet precede vasoconstriction. These findings suggest that the vascular tone in the spinal cord can be modulated by dopamine released from descending fibers *via* the activation of astrocytes.

Acknowledgement

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T15-007B

Endothelial cells and astrocytes, main component of the blood brain barrier, as new targets for the modulation of the sphingosine-1-phospate receptor

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The blood brain barrier (BBB) is involved in the initiation and maintenance of neuroinflammation, allowing, when damaged, the access of blood-borne proteins and immune cells. Recently siponimod (BAF-312), a sphingosine-1 phosphate (S1P) analog, has been approved for the treatment of secondary progressive multiple sclerosis (MS). Although BAF-312 acts mainly on leukocytes, S1P receptors are also expressed on BBB cellular elements, endothelial cells and astrocytes. Using an in vitro BBB model, where human endothelial and astrocyte cell lines were co-cultured, we investigated BAF-312 effects on barrier properties. Cultures were exposed to inflammatory cytokines (TNFα, 10UI + IFNγ, 5UI, T&I) in the presence of BAF-312 (100 nM). Changes of barrier properties induced by inflammatory challenge were blunted by BAF, as evaluated by measurement of transendothelial electrical resistance, barrier permeability to a dye-conjugated sugar and expression of the tight junction protein, claudin-5. BAF-312 acts on S1P1 and S1P5 and using selective agonists (AUY-954, 300 nM and UC-WP042, 1 μM respectively) we proved both receptors are involved in the modulation of claudin-5. S1P5 reduced the expression of MMP9, known to digest claudin-5, while S1P1, through PI3K, increased claudin-5 localization at cellular boundaries. Finally, BAF-312 reduced T&I-induced expression of the endothelial adhesion molecule ICAM-1, while in astrocytes it downregulated gene expression of chemoattractant factors, as CCL2 and IL-8, thus completely preventing PBMCs migration through the in vitro BBB. This effect was only observed when endothelial cells were co-cultured with astrocytes, thus pointing out the involvement of astrocytes in BAF-312 modulation of transendothelial migration. Our data indicate that BAF-312 modulates BBB properties reverting its impaired function during inflammation, suggesting an additional effect for this compound in the treatment of MS.

T15-008B

Role of astrocytes in development and progression of primary familial brain calcification

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Primary familial brain calcification (PFBC) is a neurodegenerative disease characterized by calcified blood vessels in the basal ganglia. Small vessel calcification in brain is also commonly observed in other neurodegenerative diseases such as Parkinson or Alzheimer's, but their pathomechanism and functional consequences are still unclear. Research in our lab is focused on understanding the pathophysiology behind occurrence of blood vessel calcification and their functional implications. Currently loss-of function mutation in six genes have been identified to underlie PFBC in humans. Four genes, namely *SLC20A2*, *XPR1*, *PDGFB* and *PDGFRB*, cause an autosomal dominant form of PFBC, while other two genes, namely *JAM2* and *MYORG*, cause bi-allelic recessive form of PFBC. Interestingly, astrocyte is the only brain cell type that expresses all these genes. Previous studies from our group have shown that vessel-calcifications elicit a conspicuous astrocytic reactivity and neurotoxic astrocyte response. We hypothesize that mutations in PFBC genes cause a cell-autonomous defect in astrocytes, which contribute to the vessel calcification and neurodegeneration. Using mouse models of PFBC, tissue imaging and proteomic and metabolomics analyses, we investigate the role of PFBC genes in astrocyte endfeet development and their impact on astrocyte homeostasis.

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T15-009B

Platelets' regulatory role on postnatal brain Neural Stem Cells of the Subependymal Zone.

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In mammals, pools of postnatal brain Neural Stem Cells (pbNSCs) reside in specialized microenvironments called stem cell niches, such as the Subependymal Zone (SEZ) of the lateral ventricles' walls. We have previously shown specific aggregation of platelets (PLTs) within the vasculature of the niche in response to a focal lysolecithin-

induced demyelinating lesion in the adjacent corpus callosum (CC) (Kazanis et al., 2015) and we have reported evidence of interaction between PLTs and pbNSCs, affecting the behaviour of the latter. This has been based on a co-culture system of SEZ-derived pbNSCs and PLTs that allows us to assess the effects of their direct cell-to-cell interaction. For example, when pbNSCs are cultured in the presence of high densities of PLTs they retain normal proliferation levels in the absence of Growth Factors (GFs). Moreover, the presence of PLTs led to increased percentages of oligodendrogenic progenitor cells (OPCs) under proliferative conditions but did not result in differentiation bias after removal of GFs. Here, we further investigate the effects of these interactions by looking at additional cell fate markers, such as Doublecortin and at apoptosis. Moreover, we have performed corpus callosum demyelination in transgenic mouse models of thrombocytopenia (Nbeal2$^{-/-}$, Crlf3$^{-/-}$) and thrombophilia (JAK2V6$^{fl/+}$) followed by extensive histological analysis of cellular and non-cellular components of the SEZ and the CC. Our results revealed deficient activation of OPCs following the lesion, without changes in neurogenesis, in thrombocytopenic mice and a significantly reduced response of the SEZ vasculature in mice with altered numbers of circulating PLTs. Notably, when co-cultures were set up using Nbeal2$^{-/-}$-derived PLTs, characterized by non-functional α-granules, both effects on pbNSCs differentiation and proliferation potential were abolished. Finally, our investigation is complemented by direct grafting of labelled PLTs in the SEZ and in the adjacent striatum. Altogether our results reveal a functional role of PLTs, as cellular entities, in the regulation of both pbNSCs and their niche, partially dependent on a-granules and their compartments.

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T15-010B

Astrocyte mechanosensitivity is amplified by albumin

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Astrocyte endfeet cover brain vasculature, forming the glia limitans that separates perivascular space from brain parenchyma and constitutes a second diffusion barrier in the neurovascular unit. Cerebral spinal fluid (CSF) from subarachnoid space enters the perivascular space (glymphatics), delivering fluid and solutes along and across the astrocyte endfeet into the brain parenchyma. Forces acting on the endfoot include changes in the intravascular (A-V) pressure due to dilation and constriction of the vessel wall, fluid shear stress along the endfoot generated by glymphatic circulation, and interstitial flow between the endfeet from basement membrane to parenchyma. Previous studies measured astrocyte Ca^{2+} responses to A-V pressures and brief concussive forces, concluding that response required relatively large forces. We have re-examined this issue applying shear stress to dissociated cortical astrocytes plated in calibrated microfluidic chambers. Flow was implemented using saline solutions (HEPES buffered-DMEM) with added albumin to mimic CSF concentrations in adults, infants, and the setting of neuroinflammation (0 to 0.1 mM) and intracellular Ca2+ was measured with the ratiometric Ca^{2+} indicator

Fura2. Cells were exposed to a range of shear stress forces (<0.1 to ~20 dyne/cm^2) for 10s or 30s via syringe pump. The experimental range of albumin concentrations was 0 to 0.1 mM. At 50μM albumin and higher, Ca^{2+} responses to both 10 and 30s stimuli did not increase further. Reponses saturated at about 3.5 dyn/cm^2, with EC50 values with 50 μM albumin about 0.8 dyn/cm^2. In the absence of albumin, Ca^{2+} response was abrogated. Number of responding cells and amplitudes of responses steeply increased in the range from 8-16 μM before saturating at 50 μM albumin. Ca^{2+} changes in astrocyte endfeet are believed to modulate vascular wall diameter and thus control blood flow in the brain. Amplification of mechanical sensitivity was blunted by treatment with 1μM fingolomid (FTY720), implying involvement of S1P receptors. Our findings that astrocytes are exquisitely sensitive to flow and that sensitivity is greatly amplified by albumin concentrations encountered in CSF in both children and adults with meningitis, hemorrhage and autoinflammatory disease predict that cerebral blood flow will be affected in these conditions and will respond to perivascular forces by augmented Ca^{2+} signaling.

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T16 | Regeneration and repair

T16-001B

Viability of rat organotypic spinal cord slices from different aged male and female rats grown under standard conditions and when subjected to in vitro injury.

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Ex vivo spinal cord organotypic culture is an advantageous model for spinal cord injury (SCI) research. It preserves *in vivo* cellular interactions and some functional properties while providing a more cost efficient, easily manipulated and ethical model of SCI than in vivo experimentation [1, 2]. *Ex vivo* models support the 3R's of animal research by replacing live models and increasing data generated from individual animals. Viability in ex vivo models is paramount to their use as experimental models, decreased viability will be less reflective of healthy tissue and thus less reliable for study.

The highest prevalence of SCI is in adult males [3]. Furthermore sex differences in SCI pathology and therapeutic responses highlight the need for SCI models to consider sex [4, 5]. *In vivo* SCI models typically use female rodents due to increased risk of urinary tract infection in male rodents after SCI. [5] *Ex vivo* models are further removed from the archetypal SCI patient favouring the use of early postnatal pup tissue rather than adult.

Postnatal animals are preferred in slice cultures due to their increased viability compared to older animals. However there are fundamental differences in the physiological properties and regenerative capacity in spinal cords at different developmental age [6]. The efficient regenerative capacity of the spinal cord in younger animals is well known, with complete regeneration after SCI observed in P2 mice [7]. This raises the questions as to how valid early postnatal tissue is as a models of SCI.

Few papers have directly compared viability of *ex vivo* slices from different aged animals. This study assessed the viability of spinal cord slice culture preparations from both male and female P4, P12 and adult (6 month old +) rats. Following previously published protocols [2, 8] spinal cords were sliced longitudinally at 350µm and cultured for 14 days total. Some slices were injured by double-scalpel blade transection (blades spaced 500µm apart) on day 4 *ex vivo* leaving 'transection gap'. Gap size and cell ingrowth was measured over days in culture as a measure of viability after injury. Ten days post injury the viability was accessed by Fluorescein diacetate (FDA) and Propidium Iodide (PI) staining. Confocal microscopy and ImageJ were used to access the percentage FDA–vs- PI stained cells.

The viability of injured and control slices will be accessed across age groups and sexes with the view to finding the most viable and representative SCI model. Ingrowth of cells into transection gap was more robust in P4 and P12 slices but not adults. Observations suggest decreased viability in adult slices compared with P4 and P12.

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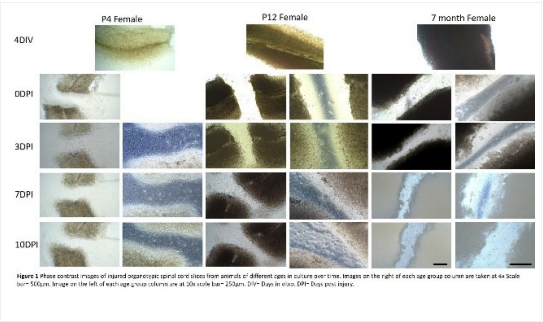
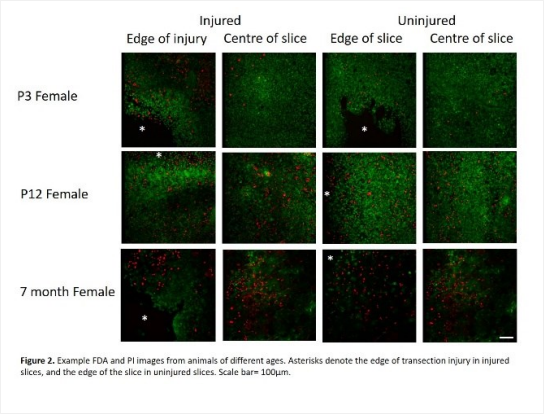


Phase Contrast Images of Spinal cord Organotypic slices in Culture

Figure 1. Phase contrast images of injured organotypic spinal cord slices from animals of different ages in culture over time. Images on the right of each age group column are taken at 4x Scale bar= 500µm. Image on the left of each age group column are at 10x scale bar= 250µm. DIV= Days in vitro. DPI= Days post injury.



FDA and PI Stained Spinal Cord Organotypic Slice Culture
Figure 2. Example FDA and PI images from animals of different ages. Asterisks denote the edge of transection injury in injured slices, and the edge of the slice in uninjured slices. Scale bar= 100µm.

T16-002B

Peripheral Nerve Maintenance after Injury and in Chronic Disease

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The peripheral nervous system (PNS) is tasked with the challenge of integrating proprioceptive and nociceptive cues to the central nervous system (CNS) and relaying outputs from the CNS to command motor function. Schwann cells, the myelinating glia in the PNS, are remarkably plastic, and can transdifferentiate in response to injury to form “repair cells,” which mediate the clearance of myelin, injured axons, and facilitate axonal regrowth. We have discovered an intrinsic pathway that guides Schwann cell transdifferentiation in both injury and disease. We found that genetic deletion of *MITF* in SCs induces no detectable developmental phenotype; however, after injury MITF is upregulated, and translocated to the nucleus to orchestrate a gene program in response to the injury. SCs that are lacking MITF aberrantly transdifferentiate after injury. This aberrant transdifferentiation in turn leads to poor axonal regrowth, as well as concomitant motor and sensory defects in vivo. Moreover, we have found that MITF upregulation is critical for nerve maintenance during certain chronic peripheral neuropathies.

T16-003B

Genomic and functional analyses reveal subtle differences between human skin and nerve derived Schwann cells

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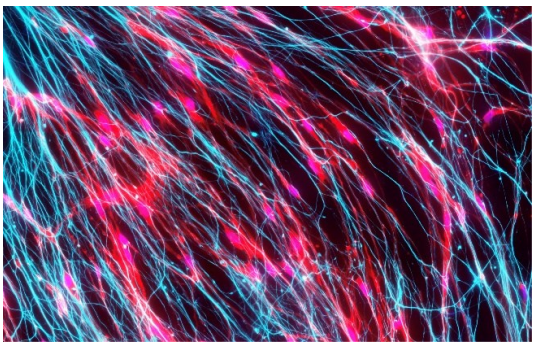
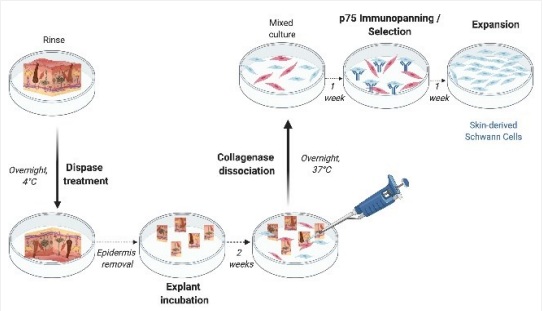
Skin is an easily accessible tissue and is a rich source of Schwann cells (SCs). We have previously shown that SCs can be selected and expanded in culture from an adult mixed skin cell population. Towards potential clinical application of autologous SC therapies, we aim to improve the reliability and specificity of our protocol to obtain SCs from small skin samples. We then subjected these cells to high resolution single cell RNA sequencing and a series of *in vitro* and *in vivo* assays to understand the differences between the skin-derived SCs and nerve- derived SCS. Our results showed that a ~1 cm^2 of human skin could yield 9-20 million SCs within 5- 6 weeks after harvest and these cells expressed classic SC makers such as S100-beta, p75, Sox10, cJun and Oct-6. Deep sequencing of skin- and nerve- derived SCs revealed close to 95% similarity in differential gene expression. Gene network analysis showed mostly overlapping profile between the two cell types but interferon regulatory factors family was significantly up-regulated in skin-SCs. In cell culture, these two cell types were similar in proliferation, migration and interaction with neurites from motoneurons differentiated from human iPSCs, either in 2D or 3D cultures. However, dorsal root ganglion (DRG) neurite outgrowth assay showed substantially more neurite extension in DRG exposed to conditioned media from skin-derived SCs compared to those from nerve-derived SCs. Examination of growth factor using ELISA revealed largely similar profiles but skin-derived SCs expressed higher VEGF whereas nerve-derived SCs expressed higher TGF-alpha. Collagen content was also significantly higher in skin-derived SC media compared to those from nerve-derived SC. Transplantation of skin- and nerve- SCs into injured tibial nerve in immunocompromised nude rats showed close association of both SCs to regenerating axons, despite a higher number of surviving nerve-derived SCs. Overall, our results showed that skin derived SCs shared almost identical properties to nerve-derived SCs but with subtle differences which could potentially complement the nerve-derived SCs in future SC therapies.

Acknowledgement

This study is supported by a CIHR Project Grant to Rajiv Midha, Jeff Biernaskie and Carol Schuurmans.



Co-culture of Schwann cells and motoneurons
Co-culture of human skin derived Schwann cells (S100, red) with human iPSC-motoneurons for a month showed alignment and close association of Schwann cells with neurites (NF200, cyan).



Isolation and purification of Schwann cells from human skin
Schematic diagram depicts isolation and purification of Schwann cells from human skin. The protocol involved epidermis removal, explant incubation, dissociation, purification using p75 antibody and expansion of skin derived Schwann cells over a period of 4-6 weeks.

T16-004B

Control chromatin remodelling enzymes in Schwann cells to improve peripheral nerve regeneration

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The regeneration capacity of the peripheral nervous system (PNS) is largely promoted by the PNS myelinating glia, namely Schwann cells (SCs). After an injury, mature SCs show a high plasticity and adopt a repair cell phenotype, which creates a favourable environment for axonal regrowth. This includes the secretion of neurotrophic factors, the degradation of the myelin sheath, and the recruitment of immune cells. Furthermore, they proliferate and migrate along the axons to form a scaffold called bands of Bungner, which provides a substrate for elongation of regenerating axons from the injury site to their previous targets. Once axon regrowth is completed, repair SCs re-differentiate into myelinating SCs and remyelinate axons. The transition from one phenotype to the other is controlled by a complex interplay between positive and negative regulators of myelination, which requires a fine-tuned regulation of their transcription. Together with transcription factors, chromatin remodelling enzymes control the phenotypic switches occurring in SCs after lesion. A previous study in our group highlighted two paradoxical functions of histone deacetylase 2 (HDAC2) during the regeneration process. On one hand, HDAC2 slows down axonal regrowth by delaying the conversion of SCs into repair SCs. On the other hand, HDAC2 acts as a positive regulator of remyelination. This study also proposes a new therapeutic strategy. Indeed, a short-term inhibition of HDAC2 early after lesion improves functional recovery. It is crucial to enhance the axonal regrowth rate after large distal PNS lesions, especially in humans because axons have to regrow over long distances. A critical time window has to be observed since chronically denervated SCs no longer support axonal regeneration. We are now focusing on another chromatin remodelling enzyme, which is strongly upregulated in SCs after sciatic nerve crush. Our aim is to identify the functions of this enzyme during the regeneration process. To this end, we carried out sciatic nerve

crush lesions in mice lacking our candidate protein specifically in SCs. Western blot, BrDU proliferation assay and electron microscopy analyses suggest a faster demyelination in the knockout mice, which may accelerate the speed of axonal regrowth. Furthermore, behavioural tests reveal a faster functional recovery. We are now studying the mechanism of action of our candidate. To this end, we will perform mass spectrometry analysis to identify potential binding partners. Chromatin immunoprecipitation and luciferase gene reporter assay will allow us to determine the target genes of our candidate protein. Taken together, a combined and short-term inhibition of HDAC2 and our new target protein may further enhance axonal regeneration and functional recovery.

T16-005B

A transcriptomic atlas of remyelination reveals diverse neural-immune states

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The process of myelinating axons after injury, remyelination, is an essential CNS regenerative function that fails in several diseases such as multiple sclerosis and amyotrophic lateral sclerosis. Successful remyelination requires a dynamic collaboration between the myelinating cells and many other cell types, including resident glia in the CNS, and circulating immune cells. However, with a few exceptions, the transcriptional diversity of these cell-types across the clearance and repair stages of remyelination is not known.

In this work, we have developed a novel single-nucleus protocol for acquisition of all cell-types, without isolation artefacts, from small frozen tissue samples. We apply this to the corpus callosum of lysophosphatidylcholine (LPC)-injected mice, which induces myelin loss and repair in a stereotyped manner over 3-4 weeks. By densely sampling single-nucleus dynamics every four days in lesion and control mice, we constructed an atlas of both the clearance and repair phases of remyelination.

Our results reveal extensive transcriptional diversity in the majority of glial cell-types, with polydendrocytes, oligodendrocytes, macrophages/microglia and astrocytes all exhibiting timepoint-dependent shifts in transcriptional states. We will outline these results and highlight the dynamics of this process, which has implications for longer-term single-cell projects which can miss gene expression changes that can occur over days.

We identified a novel macrophage population that does not express traditional markers of invading monocytes but exhibits a gene signature associated with Alzhiemer's Disease. We will outline lineage-tracing experiments to confirm the ontogeny of this new state. We also observe a repair-phase specific population of adaptive immune cells that may play a critical role by secreting cytokines and other signalling molecules. The function of these cells is under active investigation and we will describe our initial results.

Finally, we are leveraging this dataset to provide a model for glial and neuroimmune biology. To this end we are using computational approaches to identify candidate transcription factors that orchestrate these extensive transcriptional changes and systematically characterizing both the temporal dynamics and the cell-cell signaling molecules. These results will guide further downstream experiments to interrogate how CNS cells integrate and execute biological functions.

T16-006B

Investigating Schwann cell and oligodendrocyte plasticity after lesion

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The peripheral nervous system (PNS) displays a high regenerative capacity after nerve injury. This ability is due to a large extent to the high plasticity of Schwann cells (SCs), the myelinating glia of the PNS. Rapidly after injury, SCs are activated by injury-induced signals and respond by entering the repair program. Repair SCs clear axonal debris, initiate myelin breakdown and promote axonal regrowth. Finally, they remyelinate regenerated axons. In contrast, the central nervous system (CNS) shows an extremely limited regenerative capacity. After a CNS lesion, oligodendrocytes (OLs), the myelinating glia of the CNS, do not demyelinate and do not promote myelin clearance. They remain inactive or undergo apoptosis and myelin debris, which contain several myelin-associated inhibitory molecules, impair axonal regrowth. Therefore, an efficient clearance of myelin debris could be essential for axon regeneration. In the early phases after a PNS injury, before macrophage invasion, myelin clearance is initiated by SCs, through a type of selective autophagy called myelinophagy during which SCs forms a phagocytic cup-like structure which engulfs intrinsic myelin fragments into the SC cytoplasm, where they can be selectively recognized or addressed to the phagopore for the autophagic destruction. To date, myelinophagy has not been described and does not appear to occur in OLs after a CNS lesion. Therefore, by identifying differences in SC and OL reaction to injury, we aim at enhancing axon regeneration by inducing myelinophagy in mature OLs.

T16-007B

Acute Injury Activates the CRH System in NG2 Glia

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The Corticotropin-releasing hormone (CRH) is a 41 amino acid peptide which has already been found to play a major role in the regulation of the peripheral immune system. Although CRH is also widely expressed throughout the brain its possible role in the immune response of the central nervous system has not been addressed yet. The observation that stabwound injury in CRH reporter mice elicits CRH expression in cells surrounding the wound initiated a detailed characterization of this phenomenon. To this end, we inflicted microlesions in the midbrain of Crh-Cre; Ai9 mice to identify these CRH expressing cells and to analyze possible time dependent changes in their expression. By immunohistochemistry and double *in situ* hybridization we were able to identify these CRH expressing cells as NG2 glia also known as oligodendrocyte progenitor cells (OPCs), a cell type which is able to generate oligodendrocytes and has already been shown to react to brain injury. Using different CRH reporter mouse models we were able to show that CRH expression is an early reaction to the injury and that the cells increase their numbers by subsequent proliferation. Thereafter, the majority of CRH expressing NG2 glia differentiates into oligodendrocytes, connecting them to remyelination after injury. The comparison between CRH expressing and non-expressing NG2 glia around the injury site showed a difference in their kinetics, implying that

the CRH expressing cells resemble a specific subpopulation of NG2 glia. In future studies we are going to look for potential target structures and interrogate the influence of this newly found CRH system in NG2 glia in wound healing and remyelination after acute injury.

T16-008B

Histone Deacetylases 4,5 and 7 promote peripheral nerve myelination during development and remyelination after injury

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The increase in cAMP in Schwann cells, caused by the activation of Gpr126, blocks the expression of the negative regulator of myelination *c-Jun* and induces the expression of *Krox-20,* what ends up triggering the myelination of the Peripheral Nervous System (PNS). We have recently shown that increased cAMP in Schwann cells causes the nuclear translocation of HDAC4 (a type IIa HDAC), in part mediated by phosphorylation of S265 / S266 by PKA. Once inside the nucleus, HDAC4 binds to the *c-Jun* promoter forming a complex with NCoR1, and recruits HDAC3, a class I histone deacetylase. This deacetylates lysine 9 of histone 3 and blocks the expression of *c-Jun*, forcing Schwann cells to exit the cell cycle and activate the genetic program of myelination driven by *Krox-20 (Gomis-Coloma et al., 2018)*.

Here, using conditional KOs mouse models for class IIa HDACs, we show that the simultaneous elimination of three of these proteins (HDAC4/5/7) in Schwann cells causes a delay in the transition from immature to differentiated Schwann cells and produces a delay in the myelination of the peripheral nervous system during the postnatal development. Also, we show that it causes a delay in the remyelination of the axons after nerve injury. Our data suggest that the absence of these HDACs hinders the silencing of *c-Jun* and *Oct-6*, delaying the transition from the pro-myelinating and repair Schwann cells stages to the myelinating phenotype.

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T16-009B

Oligodendrocyte heterogeneity during myelin regeneration

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Demyelinating conditions such as Multiple Sclerosis (MS) are characterised by damage to and loss of myelin in the central nervous system (CNS). As no regenerative or neuroprotective therapies currently exist, a major goal is to identify strategies to enhance the regenerative capacity of the CNS. It is well established that remyelination can occur via the generation of new oligodendrocytes from resident-progenitors, but it remains unclear to what extent mature oligodendrocytes, including those that survive demyelination, can contribute to the regeneration of myelin. We use longitudinal live imaging in zebrafish to study myelin regeneration with single cell resolution of both newly generated oligodendrocytes and those that survive demyelination. We show that newly generated oligodendrocytes are far more efficient at regenerating and targeting myelin than those that survive demyelination. We aim to define the molecular and cellular nature of oligodendrocyte heterogeneity during remyelination in zebrafish, and also in humans, through the analysis of MS tissue, in order to identify strategies to promote remyelination for the treatment of human disease.

T16-010B

Schwann cell reprogramming for improved nerve repair

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Schwann cells are glial cells whose main function is to myelinate peripheral nerve axons. They are necessary for peripheral nerve repair. However, endogenous Schwann cell repair capacity is often inadequate due to chronic injury, disease or aging. A solution is to engineer a renewable source of Schwann cells with enhanced repair properties for transplantion into an injured nerve. For this purpose, we are developing a protocol to trans-differentiate fibroblasts or pluripotent stem cells into 'repair' Schwann cells. *Sox10* is a master regulator of a Schwann cell fate, and can reprogram adult fibroblasts into a Schwann cell identity. After nerve injury, Schwann cells re-express several embryonic glial linage genes, which we used to engineer two triple transcription factor (TF) expression vectors (TTFEVs) that include *Sox10* and embryonic repair genes (*Sox10-Jun-Sox2, Jun-Pax3-Sox2*). TTFEVs carrying these combinations, as well as *GFP* and *Sox10* only controls, were transfected into embryonic d14 mouse embryonic fibroblasts (MEFs) and grown in media conducive to Schwann cell differentiation. The expression of TTFEV gene relative transcripts and other Schwann cell markers was analysed on days 14 and 21 after transfection using qPCR and immunostaining. The TTFEVs induced higher levels of expression of Schwann cell markers. We are now testing the ability of our TTFEVs to convert human pluripotent stem cells into 'repair' SCs, using a self-replicating mRNA approach to increase expression levels. This project will lead to the development of a cell-based therapeutic that can be transplanted into the injured nerve of patients after peripheral nerve injury.

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T16-011C

The matricellular protein Connective Tissue Growth Factor promotes motor axon regeneration

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The peripheral nervous system (PNS) relays signals between the central nervous system (CNS) and the rest of the body. At variance from the CNS, the PNS has the ability to repair and regenerate to a certain extent, thanks to intrinsic abilities of motor neurons, and to a permissive environment. Nevertheless, peripheral nerve injuries represent an important clinical issue, as neuromuscular function rescue is often not complete, and may lead to permanent disabilities. Regeneration of the neuromuscular junction (NMJ) is orchestrated by signals exchanged among its components, i.e. the motor axon terminal, perisynaptic Schwann cells (PSCs) and the muscle. PSCs are surrounded by a basal lamina, a specialized extracellular matrix which regulates PSCs proliferation, survival, migration, and supports peripheral nerves. Desite many efforts, the molecular determinants of peripheral nerve regeneration are largely unknown.Neuronal hydrogen peroxide (H_2O_2), produced in response to a nerve injury, is a major Schwann cells (SCs) activator. Genes differentially expressed by SCs exposed to H_2O_2 were used here as a signature gene set for a functional enrichment analysis of NMJ transcripts profiled during motor axon terminal degeneration and re-growth by the presynaptic neurotoxin α-latrotoxin (α-Ltx). This neurotoxin, isolated from the black widow spider venom, targets specifically the motor axon terminal causing its complete but reversible degeneration. We found that at the regenerating NMJ: i) the H_2O_2 signature is enriched in extracellular matrix terms; ii) the mRNA of Connective Tissue Growth Factor (Ctgf) is strongly up-regulated, iii) Ctgf is produced by terminal SCs, iv) Ctgf neutralization delays functional recovery upon nerve injury. In conclusion, the present study provides compelling evidence that: 1) the transcriptome of the regenerating NMJ is a powerful source of candidates with pro-regenerative potential and 2) for the first time in mice, that Ctgf haspro-regenerative action upon peripheral nerve injury. These findings may have high potential therapeutic value for the improvement of functional recovery after various types of nerve insults, including mechanical traumas, genetic neurodegenerative syndromes and in spinal cord diseases.

T16-012C

Heparan Sulphate mimetics as a therapeutic for central nervous system repair

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Demyelination and axonal pathology in the central nervous system (CNS) is associated with numerous neurological disorders such as spinal cord injury (SCI) and multiple sclerosis (MS). Any repair strategies for CNS damage must have a multifactorial approach including promotion of axonal outgrowth, and remyelination. One such candidate is heparin sulphate mimetics which are glycomolecules of repeating disaccharide units synthesised from heparin (mHeps) to express varying levels of sulphation and lack coagulation activity. They share structural similarities to cellular heparan suphates (HS) which modulate a huge range of cellular function. They are thought to regulate a variety of cell signalling by both sequestering ligands and acting as a cofactor in the formation of ligand-receptor complexes. Using these mimetics, we have demonstrated that low sulphated mimetics (LS-mHeps) enhance neurite outgrowth and myelination *in vitro* by sequestering molecules that inhibit myelination. Thus, LS-mHeps have the capacity to represent novel candidates as therapeutics for CNS damage. Here, we test our lead compound, in an ex vivo and in vivo model namely experimental autoimmune encephalomyelitis (EAE) and mouse cerebellum slice cultures. LS-mHep7 treated EAE animals recovered faster and had reduced numbers of inflammatory cells within spinal cord lesions. Interestingly, LS-mHep7 promoted a more rapid restoration of pre-disease animal weight and promoted appetite directly in non-diseased animals. This suggests LS-mHep7 has beneficial effects on both animal welfare and appetite. Ex vivo data shows similar promotion of myelination supporting the future clinical translation of these next generation heparin mimetics as a novel treatment for CNS diseases.

Acknowledgement

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T16-013C

The pharmacological co-activation of Smoothened and the androgen receptor results in an unexpected therapeutic cooperation in models of central nervous system demyelination

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In the central nervous system (CNS), the Hedgehog signaling pathway has been implicated in oligodendrocyte and myelin production in several demyelinating models while androgens and their main receptor, the androgen receptor (AR), were found to play a key role in spontaneous regeneration of myelin occurring upon CNS demyelination. Here, we have investigated the existence of a possible cooperation between the Hedgehog and androgen pathways in the context of remyelination. We show that the activation of each pathway unexpectedly induces both the proliferation and the differentiation of oligodendrocyte progenitor cells (OPCs). We propose that the pharmacological activation of Smoothened (Smo), the key transducer of Hedgehog signaling, directly promotes OPC proliferation, but indirectly induces OPC differentiation by promoting microglia polarization toward an anti-inflammatory phenotype previously shown to drive oligodendrocyte differentiation during remyelination. On the other side, testosterone appears to stimulate OPC proliferation without requiring its aromatase-mediated

conversion to estradiol whereas testosterone-mediated OPC differentiation requires aromatase activity. Hedgehog and androgen co-activation results in accelerated myelin regeneration possibly related to the respective role of each drug on the response of microglia and astrocytes to demyelination. The functional cooperation of the pathways is not limited to myelin repair as suggested by the higher preservation of axon integrity, the lower neuroinflammation and the quite complete regression of neurological disabilities observed in the experimental autoimmune encephalomyelitis model of demyelination. From a molecular point of view, the combined drugs uniquely induce a strong decrease of several deleterious cytokines including GM-CSF, TNF-α and IL-17A in the demyelinated spinal cord, which may break the vicious circle leading to sustained neuroinflammation since GM-CSF produced by encephalitogenic T cells, causes the production of active IL-1β through inflammasome activation in a subpopulation of monocytes. Our data also suggest the existence of a regulatory loop involving the dendritic cells and resulting in the synergistic control of GM-CSF and IL-2 production. Both improvement of myelin regeneration and lowering of neuroinflammation are likely important to consider in males who are at higher risk than females to develop secondary progressive multiple sclerosis and for whom low testosterone levels are correlated with disease severity. Our data should open therapeutic perspectives that might break the vicious circle inflammation-demyelination-neurodegeneration that up to now cannot be halted by treatments currently available for patients suffering from multiple sclerosis.

T16-014C

Characterising the Schwann cell early injury response in mouse and zebrafish

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After peripheral nervous system (PNS) injury, axons degenerate using a programmed axonal death pathway, and Schwann cells adopt a repair phenotype. Both processes are regulated by cell intrinsic molecular pathways. The Sterile- α and Toll/interleukin 1receptor (TIR) motif containing protein 1 (SARM1) is a central activator of axon degeneration and the transcription factor c-JUN is a key regulator of repair Schwann cells [1,2]. Repair Schwann cells digest myelin using myelinophagy, attract macrophages, support the survival of damaged neurons and their growth and guidance to their targets. The Schwann cell injury response is, however, incompletely understood and early injury responses have not been fully characterised spatially and temporally [3]. Furthermore, it is uncertain whether early Schwann cell injury responses rely on activation of the axon-intrinsic degeneration pathway.

To interrogate this problem, we used a number of techniques. Firstly, we performed RNA sequencing of distal tibial nerves after proximal injury at the sciatic notch in *wild-type* and *Sarm1* knockout mice, at multiple early timepoints prior to the time of axon degeneration. Secondly, we developed an *in vitro*, murine neuron/Schwann cell myelinating coculture system in microfluidic chambers to image axon-Schwann cell interactions after axotomy. Finally, we live imaged an *in vivo* larval zebrafish model of peripheral nervous system injury using 2-photon axotomy of the posterior lateral line (PLL) nerve.

Results show that there are substantial gene expression changes in *wild-type* distal tibial nerves, a location very remote from the injury site, at time points prior to significant axon degeneration. These gene expression changes did not occur in *Sarm1* knockout distal tibial nerves. Importantly we show that *Sarm1* is not required for CNS or PNS myelination in mouse and zebrafish or cell autonomously for the Schwann cell injury response. Lastly, we further characterise the PLL 2-photon laser axotomy model in larval zebrafish, timing axon degeneration and Schwann cell injury gene expression profiles, using third generation in situ hybridisation chain reaction.

Overall, this study supports a model of Sarm1-dependent axon injury signalling to Schwann cells. This deepens our understanding of axo-glial signalling in PNS injury and will hopefully lead to the identification of new pathways that can modulate the phenotype of repair Schwann cells for therapeutic effect in neuropathy and nerve repair.

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T16-015C

Ageing Alters Pericytes Response to Demyelination – Consequences for Myelin Repair?

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Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS). Although remyelination represents a robust regenerative response to myelin damage, it largely fails in late MS stages. MS has an onset in the young population; however, it progresses throughout life. Ageing hampers oligodendrocyte progenitor cells (OPCs) function leading to a decreased number of newly-formed oligodendrocytes and to an impaired remyelination. We have previously shown that PDGFRb-expressing pericytes (PCs) response to demyelination, proliferate and are found in close proximity to differentiating OPCs [1]. Furthermore, PCs secrete soluble factors, such as Lama2, that promote OPC differentiation as well as instruct an oligodendrocyte fate choice in adult neural stem cells (NSCs) [2], supporting the generation of oligodendrocytes and myelin repair. Here, we aim to determine whether ageing alters PCs response to demyelination. Following lysolecithin-induced focal demyelination in the caudal cerebellar peduncle of young (2 months old) and aged (more than 17 months old) rats, we evaluate PCs proliferation as well as their localisation and numbers at 5-, 14- and 21-days post lesion. We found a decrease in PCs proliferation in response to myelin damage leading to a significant reduction in PCs numbers during remyelination in aged rats. We are currently evaluating whether ageing affects the ability of PCs to

promote the generation of oligodendrocytes from OPCs and NSCs. Our findings indicate that ageing alters PCs response to demyelination and suggest that it may also affects PCs contribution to myelin repair, but this needs to be further study.

Acknowledgement

Funding from Chilean Agencia Nacional de Investigación y Desarrollo (ANID) - Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) Regular Grants Nº 1201706 and Nº 1161787, Programa de Colaboración Internacional (PCI) Grants Nº 170233 and Nº 180139.

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T16-016C

The role of class IIa HDACs in Aged Schwann cells.

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Nerve regenerative capacity is hampered in aged animals by a poorly understood mechanism. Repair Schwann cells are necessary for peripheral nerve regeneration and remyelination after nerve damage. It is known that after injury there is an upregulation of c-Jun, a transcription factor pivotal for the switch to the repair Schwann cell phenotype. It has been recently reported that c-Jun induction is lower in the aged nerves, what causes defects in the activation of the repair Schwann cell phenotype delaying axonal growth. Class I Histone Deacetylases (HDACs) play a central role in myelin development and the maintenance. Among them, HDAC3 has been shown to be important for maintaining myelin homeostasis in aged nerves. The aim of this work is to study if class IIa HDACs are also involved in the myelination of aged nerves. To this aim, we explore if there was any difference in myelin homeostasis and remyelination after injury in aged mice lacking HDAC4, HDAC5 and HDAC7.

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Wagstaff, Gomez-Sanchez, et al. eLife 2021;10:e62232.

T16-017C

Direct conversion of adult rat olfactory ensheathing glia (OEG) into neurons, with small molecules.

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Olfactory ensheathing glia (OEG) is a unique cellular type that surrounds growing axons of sensory olfactory neurons. Previous studies have shown that these cells are able to promote in vitro axonal regeneration of adult neurons from the central nervous system (CNS). In vivo, they can induce functional recovery through axonal repair/reconnection in animal models of spinal cord injury. If we add to OEG regenerative capacity, the possibility of converting some of these cells into neurons, we would considerably improve the recovery of the damaged area.
Cellular reprogramming consists in generating a differentiated cellular type, from a somatic cell. Direct conversion is a direct cellular reprogramming in which the cell does not pass through a pluripotent state and it is commonly achieved through the expression of one or more transcription factors. In order to avoid cellular genetic manipulation, an alternative approach to accomplish cellular reprogramming is the use of small molecules that target signalling pathways.
In this work, we present the results of reprogramming adult rat OEG into neurons –induced neurons-, using a cocktail of small molecules. Induced neurons have been characterized by analyzing the expression of neuronal markers, such as Tuj1 and SMI31 (Figure 1).

Acknowledgement
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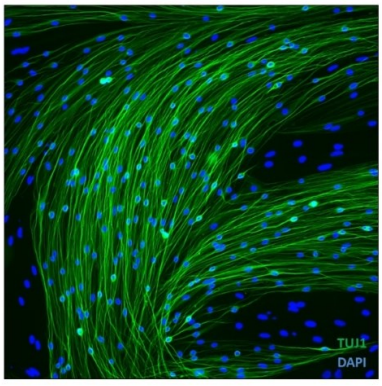

Figure 1.Direct reprogramming of adult rat OEG into neurons.
Tuj 1 positive induced neurons, from adult rat OEG, using a cocktail of small molecules.

T16-018C

Direct conversion of adult human olfactory ensheathing glia (OEG) into neurons

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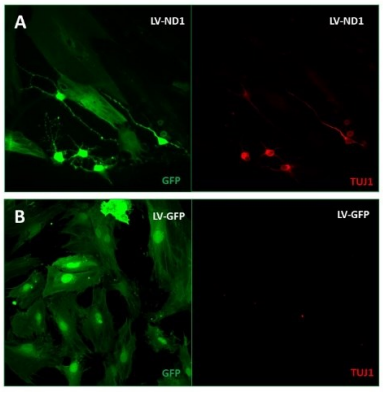
Olfactory ensheathing glia (OEG) is a unique cellular type that surrounds growing axons of sensory olfactory neurons. Previous studies have shown that these cells are able to promote in vitro axonal regeneration of adult neurons from the central nervous system (CNS). In vivo, they can induce functional recovery through axonal repair/reconnection in animal models of spinal cord injury.

Cellular reprogramming consists in generating a differentiated cellular type, from a somatic cell, through the expression of one or more transcription factors. Direct conversion is a direct cellular reprogramming in which the cell does not pass through a pluripotent state.

If we add to OEG regenerative capacity, the possibility of converting some of these cells into neurons, to bridge the gap of the injury, we would considerably improve the recovery of the damaged area. In order to achieve that goal we will make use of cellular reprogramming techniques.In this work, we present the results of reprogramming adult human OEG into neurons –induced neurons-, through the expression of the transcription factor NeuroD1. Induced neurons have been characterized by analyzing the expression of general and mature neuronal markers, such as Tuj1 and Synapsin I.



Direct reprogramming of adult human OEG into neurons
Adult human OEG infected with lentivirus-NeuroD1 (A) and lentivirus-GFP (B) expressing neuronal marker Tuj1.

T16-019C

A zebrafish model to investigate the cellular mechanisms of neurodegeneration following demyelination

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Multiple Sclerosis is a complex, heterogenous disease characterised by demyelination and neurodegeneration of the central nervous system. Loss of myelin around axons, leading to their subsequent degeneration is the primary driver of clinical progression in patients. However, we still know relatively little about the mechanisms underlying this pathological process in MS, a fact which is highlighted by the lack of available treatments aimed at preventing neurodegeneration and therefore progression of the disease.

To better understand the process of neurodegeneration following demyelination in vivo, we have utilised the optically transparent zebrafish, a model which enables whole neuron imaging over time in a living animal. To induce demyelination, we have generated a stable, transgenic zebrafish model (mbp:TRPV1-tagRFPt) that expresses a capsaicin-sensitive TRPV1 channel under the myelin basic protein promoter allowing selective expression in myelinating glial cells. We have shown that following a short, single treatment with the drug capsaicin, activation of TRPV1 channels on myelinating oligodendrocytes results in extensive demyelination with an >80% reduction in myelin 24 hrs later. Through single cell time-course and electron microscopy analysis we have shown that there is an active repair process following demyelination where axons are successfully remyelinated by both newly differentiated and existing oligodendrocytes. To investigate the potential pathological responses of neurons and axons in the days following demyelination, we have used a variety of transgenic reporters to visualise and follow the time-course of individual axons during de- and remyelination. Through this, we have identified imaging measures of neurodegeneration and tested if these can be reversed through chemical manipulation with the premise that promoting remyelination of axons will provide neuroprotection.

The zebrafish TRPV1 demyelination model we present here provides not only a useful model for investigating the mechanisms underlying neurodegeneration in vivo but also a novel, drug screening platform to evaluate the potential of neuroprotective therapeutics of relevance to MS.

T16-020C

Reduction of microglia proliferation after spinal cord injury in mice and nonhuman primates improves functional recovery

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No curative treatment is available for any deficits induced by spinal cord injury (SCI). Following injury, microglia undergo highly diverse activation processes, including proliferation. In this study, we investigated whether microglial proliferation is beneficial or detrimental for recovery following SCI. In the intact central nervous system, principally microglia express the macrophage colony stimulating factor-1 receptor (CSF1R). Here we report that a transient (1-week) oral administration after SCI of GW2580, an inhibitor of CSF1R that inhibits microglial proliferation, improves motor function recovery, and promotes tissues preservation and/or reorganization in mice. To progress toward translational research, we then orally administrated GW2580 over 2 weeks after SCI in nonhuman primates. Similar to mice, transient CSF1R inhibition improves both static and dynamic parameters of motor function recovery, and promotes myelin preservation identifiable by Coherent anti-stokes Raman scattering microscopy. Our findings suggest that a transient depletion of microglia proliferation early after SCI reduces tissue damage and improves motor function recovery in both mice and nonhuman primates. Thus, GW2580 treatment may provide a promising therapeutic option in SCI.

T16-021D

Enhancing remyelination with CRISPR/Cas9 edited human oligodendrocyte progenitor cells

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In Multiple sclerosis (MS) remyelination can happen, but is often insufficient. Examination of human post mortem tissue from MS patients shows that one of the reasons that remyelination is insufficient is that 30% of demyelinated lesions, particularly chronic lesions, contain reduced numbers of oligodendrocyte progenitor cells (OPCs) (Boyd et al.,2013), which are one contributor to remyelination by their maturation into myelin-forming oligodendrocytes. Single nuclei RNA-sequencing data also supports a lack of OPCs in MS tissue (Jaekel et al., 2019). This may be related to high levels of chemorepellent factors such as Sema3A in chronic MS lesions. Enhancing OPC migration into these lesions is therefore of therapeutic interest to promote remyelination and neuroprotection. Recent work, however, suggests that OPCs and oligodendrocytes in MS brains may be dysfunctional, senescent or prematurely aged (Nicaise et al., 2019) and that targeting the endogenous cell population may not be successful. It may instead be preferable to transplant exogenous 'improved' OPCs, which are, for example, genetically edited to be unresponsive to the chemorepellent environment.

To test this, we first generated a clinical grade human embryonic stem cell line (hESC) constitutively expressing a membrane targeted green fluorescent protein (GFP) tag. As a proof-of-principle, using CRISPR/Cas9, we then chose to knockout the neuropilin1 receptor (NRP1) which is the exclusive receptor of Sema3A and is expressed in OPCs, aiming to generate human GFP+NRP1- OPCs that are unresponsive to the chemorepellent Sema3A and therefore better recruited to demyelinated lesions. These genetic modifications did not affect the cells differentiation capacity with both GFP+ and GFP+NRP1- hESCs generating oligodendroglia *in vitro* and myelin sheaths *in vivo* following transplantation into *Shiverer:Rag2*$^{-/-}$ mice. To assess the migration, survival and remyelination capacity of these cells *in vivo*, a focal demyelinated lesion was generated in adult *Rag2*$^{-/-}$ mice. When hGFP+OPCs were introduced directly into such lesions, good cell survival was observed and after 8 weeks had contributed to lesion remyelination. Cell migration was assessed by injecting cells 1mm contralateral to the lesion and examining their recruitment to the lesion. This was further quantified in a chronic lesion model in which focal lesions are loaded with Sema3A and with the GFP+NRP- hOPCs. This data will generate proof-of-principle information about genetically editing human OPCs to enhance remyelination.

T16-022D

Exposure to fine particulate matter (PM) hampers myelin repair in a mouse model of white matter demyelination

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Epidemiological studies show a strong association between exposure to air pollution – and particularly to particulate matter (PM) - increased prevalence of Multiple Sclerosis (MS) and higher rates of hospital admissions for MS and MS relapses. Beyond having immunomodulatory effects and sustaining a systemic oxidative-inflammatory response, PM may participate in MS pathogenesis by targeting also Central Nervous System (CNS)-specific processes, such as myelin repair. Here we show that, in a mouse model of lysolecithin-induced demyelination of the subcortical white matter, post-injury exposure to fine PM hampers remyelination, disturbs oligodendroglia differentiation dynamics and promotes astroglia and microglia reactivity. These findings support the view that exposure to fine PM can contribute to demyelinating pathologies by targeting the endogenous regenerative capability of the CNS tissue.

Acknowledgement
Our work was supported by FISM - Fondazione Italiana Sclerosi Multipla – Project cod. 2019/PR-Multi/003 to Enrica Boda and Valentina Bollati, and financed or cofinanced with the '5 per mille' public funding. This study was also supported by Ministero dell'Istruzione, dell'Università e della Ricerca-MIUR project "Dipartimenti di Eccellenza 2018–2022" to Department of Neuroscience "Rita Levi Montalcini".

T16-023D

MMP2 as a possible modulator of the inflammatory response during axonal regeneration in the mouse visual system

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Despite intensive research, induction of axonal regeneration and functional recovery of the damaged central nervous system (CNS) remain a challenge. Although neuroinflammation has been put forward as a mechanism to trigger axonal regrowth in the CNS, little is known about the underlying cellular and molecular players linking inflammation to successful axonal regeneration. In this context, we disentangled the intriguing interplay between matrix metalloproteinases (MMPs), more specifically MMP2, and inflammatory processes during axonal regeneration using an optic nerve crush (ONC) combined with inflammatory stimulation model (ONC+IS) (regeneration model).

Besides the well-known role of MMP2 in clearing the path for axons to regrow during axonal regeneration, we provide further evidence for the importance of MMP2 in *in vivo* inflammation-induced axonal regeneration. Therefore, *Mmp2*$^{-/-}$ mice and wild-type littermates were subjected to an ONC+IS and axonal regeneration was assessed by quantifying fluorescently labeled cholera toxin beta positive axons in optic nerve sections. *Mmp2*$^{-/-}$

mice show reduced axonal regeneration compared to WT mice after ONC+IS, suggesting MMP2 is beneficial for axon regrowth. Three putative ways by which MMP2 could facilitate axonal regeneration were further investigated. First, using immunohistochemistry (IHC) on retinal whole mounts, we disclosed that MMP2 deficiency does not affect retinal ganglion cell survival. Of note, we identified Müller glia and myeloid cells as major producers of MMP2 after ONC+IS using IHC on retinal and optic nerve sections. In order to unravel the importance of the myeloid cells producing MMP2, heterologous bone marrow transplantations were performed. These show a partial rescue of the *Mmp2*$^{-/-}$ phenotype, suggesting that MMP2 produced by innate immune cells is needed for axonal regrowth. These data imply a more pleiotropic role for MMP2 than initially thought. Thus second, flow cytometry revealed that *Mmp2*$^{-/-}$ mice have similar numbers of the different myeloid cell populations in the retina and optic nerve after ONC+IS, indicating MMP2 does not affect the influx or proliferation of myeloid cells. Third, *Mmp2*$^{-/-}$ mice did show a reduced pro- and increased anti-inflammatory cytokine expression profile in the retina in comparison to WT mice, suggesting MMP2 stimulates, either directly or indirectly, the upregulation of pro-inflammatory cytokines and suppresses the upregulation of anti-inflammatory cytokines in the retina.

Although additional studies are needed to expand our insights in the exact working mechanisms of MMP2 during inflammatory-induced axonal regeneration, our findings provide further evidence for MMP2 as a possible modulator of the inflammatory response during axonal regeneration.

T16-024D

Profiling the molecular signature of Satellite Glial Cells in healthy and diseased states reveals key pathways for neural repair

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Sensory neurons with cell bodies in dorsal root ganglia (DRG) have been used extensively as a model to study axon regeneration in the peripheral nervous system. For decades, peripheral axon regeneration was focused on the intrinsic neuronal response to injury or the Schwann cells surrounding the axon. However, sensory neurons reside within a highly complex microenvironment in the DRG composed of multiple cell types. We thus determined the contribution of non-neuronal cell types comprising the peripheral sensory ganglia in axon regeneration. We focused on Satellite Glial Cells (SGC), a poorly studied population of glial cells in the peripheral nervous system, which completely envelop the neuronal soma. Using a single cell transcriptional approach, we determined that SGC are transcriptionally distinct from Schwann cells and share similarities with astrocytes. We also found high similarities between rodent and human SGC, especially in pathways related to lipid metabolism and PPAR signaling. By comparing the response of SGC to peripheral (sciatic nerve crush) and central injuries (dorsal root crush and spinal cord injury), we uncovered that SGC play a previously unrecognized role in peripheral nerve regeneration (1). Specifically, we discovered that PPARα activity downstream of fatty acid synthesis in SGC contributes to promote axon regeneration in adult peripheral nerves (2). Activation of the PPAR signaling pathway in SGC did not occur after central injuries. Treatment with the FDA-approved PPARα agonist fenofibrate, which is used to treat dyslipidemia, increased axon regeneration after dorsal root injury. This work highlights that the

sensory neuron and its surrounding glial coat form a functional unit that orchestrates nerve repair and that manipulation of SGC could lead to avenues to promote functional recovery after nervous system injuries. Beyond nerve injuries, we also determined the molecular signature of SGC in disease states, including autism. Our results highlight that SGC may contribute to diseases related to neurodevelopment. Ongoing studies are investigating the molecular mechanisms through which SGC regulate neuronal function and dysfunction in disease models.

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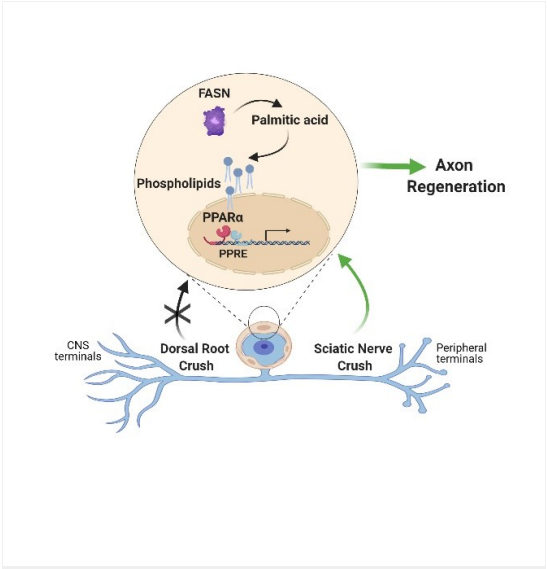

Model describing the role of SGC in peripheral nerve regeneration
Peripheral, but not central injury activates the FASN-PPARa signaling axis in SGC to promote axon regeneration

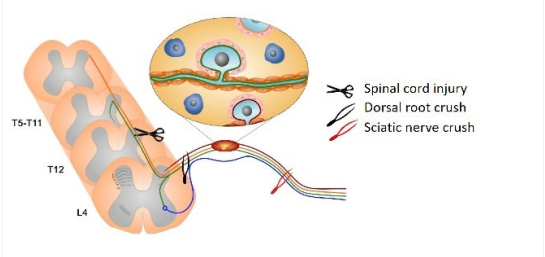

Diagram of mouse peripheral and central injury models used for genetic profiling of SGC

T16-025D

Morphometric analyses of Clonally-Related Cortical NG2-Cells in Different Multiple Sclerosis Brain Model's Scenarios

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NG2-cells, also known as oligodendrocyte precursor cells (OPC), are a heterogeneous glial cell population based on their morphology, location, molecular expression, and function. The basis of such is still under debate and whether it can be driven by intrinsic factors, such as their ontogenic origin, remains unclear. At this respect, we recently showed the different clonal responses in experimental autoimmune encephalomyelitis (EAE) mice, revealing that groups of NG2-glia clones display a heterogeneous response to brain damage and perivascular infiltration (Barriola et al., 2020). Now, we sought to analyze the relationship between both their morphology and ontogeny in different Multiple Sclerosis (MS) scenarios. To address this question, we integrated a genetic lineage-tracing method, StarTrack, along with a morphometric cluster analysis, and compared two MS mice models, EAE and Cuprizone, with control brains. Hence, we correlated the level of reactivity with different morphometric parameters measured in the derived NG2-cell progeny of StarTrack targeted single progenitors. Then, we defined different subgroups of NG2-cells based on the quantification of different morphological parameters. Data from the hierarchical cluster analysis allowed us to unravel different NG2-cells clusters categorized by morphological parameters related not only to their ontogenic origin, but also to their changes in different demyelinating scenarios. In summary, a better understanding of NG2-glia heterogeneity is relevant to deciphering the physiological role of these cells both in the healthy brain and in response to disease.

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T16-026D

Investigation of IL-22 in CNS regeneration

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Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system (CNS), causing myelin loss and death of myelin-producing cells, oligodendrocytes. The resulting neurodegeneration causes an

accumulation of disability and progressive decline in patients, in part due to a failure of remyelination. Although implicated in the pathogenesis of MS, $CD4^+$ T cells are also required for CNS remyelination. IL-22, a $CD4^+$ T cell-secreted cytokine, has shown regenerative properties in the liver, intestine and thymus, and has shown protective effects in the CNS. The endogenous IL-22 antagonist, IL-22 binding protein (IL-22BP), is a known MS risk gene and IL-22BP$^{-/-}$ mice show a less severe EAE course, which is IL-22-dependent. However, the mechanism of IL-22-mediated CNS protection and potential roles in regeneration are still unknown. I hypothesise that IL-22 has regenerative effects in demyelinating diseases.

To study the effects of IL-22 on CNS cell cultures, mixed glial cells, oligodendrocyte progenitor cells, and microglia were treated with recombinant IL-22. Microglia were then incubated with latex beads and myelin debris to assess the impact of recombinant IL-22 on phagocytosis. Recombinant IL-22 had no significant effect on proliferation or differentiation of oligodendrocyte progenitor cells nor on microglial phagocytosis.

To study the role of IL-22 in the processes of demyelination and remyelination *in vivo*, lysolecithin-induced demyelinating lesions from mice were immunofluorescently stained for components of the IL-22 receptor family. IL-22 receptor (IL22RA1) was expressed within demyelinating lesions, particularly by microglia at 5 days post lesion. However, this receptor did not co-localise with the partnering signalling component, IL-10Rβ, which was expressed by astrocytes.

The observed expression of IL22RA1 following CNS demyelination at the peak time-point of myelin phagocytosis and oligodendrocyte progenitor cell differentiation, did not correlate with significant effects of recombinant IL-22 on proliferation or differentiation in glial cell cultures neither did it impact on microglia phagocytosis. The lack of co-localisation of IL22RA1 and IL10Rβ suggests that IL-22RA1 does not accurately reflect expression of the IL-22R complex in demyelinated lesions. Future studies will address the role of IL-22 in *ex vivo* tissue modelsduring myelination, demyelination and remyelination, to fully elucidate the potential role of IL-22 in CNS regeneration.

T16-027D

Functions of histone demethylases in developmental myelination and remyelination after lesion

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The multilamellar myelin sheath, produced by Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS), represents a prominent structural feature of the nervous system and is crucial for its function and integrity. The importance of myelin becomes apparent in various neurological diseases such as Charcot-Marie-Tooth or multiple sclerosis where demyelination, dysmyelination or hypomyelination have been identified as major features. Regeneration capacities of the PNS and the CNS differ substantially. While, upon axonal injury, Schwann cells are able to demyelinate and convert into repair cells that stimulate axonal regrow and build up new myelin, oligodendrocytes in the CNS basically remain inactive at the site of injury or die and axons are not able to regrow, partly due to inhibitory factors in the uncleared oligodendrocyte myelin. It is therefore of utmost important to understand how myelination, demyelination and the conversion into repair cells are controlled in Schwann cells and oligodendrocytes in order to be able to induce or enhance nervous system regeneration.

The transcription factor Sox10 is critical for both PNS and CNS myelination and remyelination and its expression levels in Schwann cells display plasticity correlating with demyelination and remyelination phases after a PNS lesion. A major focus of this project is to investigate how histone methylation regulates the activation of the *Sox10*

gene and thus Sox 10 expression. We found that histone demethylases are regulated in Schwann cells in response to a sciatic nerve lesion and in oligodendrocytes after a demyelinating lesion, and that they are involved in the regulation of Sox10 levels in Schwann cells and oligodendrocytes. We are now investigating the mechanism of action of these enzymes and how they affect Sox10 expression and (re)myelination.

T16-028D

Repurposing of edaravone as myelin regenerative drug: chemical and biological characterization of novel small molecule derivatives

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Multiple sclerosis (MS) is a chronic inflammatory, demyelinating disease of the central nervous system characterized by oligodendrocyte loss and axonal pathology. Progressive degeneration and accumulation of disability in MS are associated with a failure in the process of damage repair and, at present, no therapy has demonstrated clinically significant regenerative properties. Edaravone is a free radical scavenger, which has been approved as a neuroprotective agent for amyotrophic lateral sclerosis in Japan, USA and Canada. We have previously shown that edaravone is able to stimulate oligodendrocyte differentiation *in vitro* and to increase the rate of remyelination in an *ex vivo* model of demyelination. In this study, we aim to evaluate the chemical-physical and biological properties of novel edaravone derivatives in mouse oligodendrocyte progenitor cells (OPC) for a better comprehension of the molecular mechanisms linked to the drug remyelinating effect. We performed the design, synthesis and biological evaluation of several analogues by exploring different substitutions on edaravone scaffold in both the phenyl ring and pyrazole. By *in silico* analysis, we selected a set of derivatives that either maintained or drastically lost edaravone properties such as lipophilicity, permeability and radical scavenger activity. The commercial analogue EDA-COOH was included in the analysis for its high antioxidant structural features. Compound properties were then confirmed in liposome-based, free radical scavenging (DPPH) and oxidative damage protection (AAPH in live cells) assays. As the stimulatory effect of edaravone on oligodendrocyte differentiation results in increased cell metabolism, we next evaluated the biological activity of all molecules in mouse OPC by the MTT enzyme reduction test. The results of these experiments showed that edaravone remyelinating activity is linked to its ability to enter biological membranes by passive diffusion distributing to both the cytosol where radicals are primarily generated, and cell membranes where lipid peroxidation proceed by radical chain reaction. In addition, since edaravone radical scavenger activity cannot fully account for its pro-differentiative effect, we are investigating the potential for the drug to interact with biological targets involved in oligodendrocyte development.

Overall, these experiments will support the repurposing of edaravone as compound with myelinating activity in the treatment of MS and other demyelinating diseases.

T16-029D

ApoA-I mimetic peptide 5A boosts remyelination by promoting myelin debris clearance

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Failure of remyelination underlies the progressive nature of demyelinating diseases. Recently, we and others demonstrated that impaired remyelination ensues in part due to a dysfunctional innate immune response in the central nervous system (CNS). Sustained accumulation of myelin-derived lipids and formation of lipid droplets, combined with an inability to process and export these lipids, was found to induce a disease-promoting phagocyte phenotype. Here, we find that the apoA-I mimetic peptide 5A, a molecule well-known to promote the stabilization and activity of the lipid efflux transporter ABCA1, markedly enhances remyelination in the cerebellar brain slice and cuprizone models. Guided by immunohistochemical and lipidomics analysis, the pro-regenerative impact of peptide 5A was attributed to increased uptake of remyelination-inhibiting myelin debris through the fatty acid translocase CD36. On a transcriptional level, peptide 5A controlled CD36 expression through the ABCA1-JAK2-STAT3 signalling pathway.Collectively, our findings indicate that peptide 5A promotes the induction of a repair-permissive environment by stimulating the clearance of inhibitory myelin debris, potentially having broad implications for therapeutic strategies aimed at promoting remyelination.

T16-030D

Harnessing the regenerative potential of cortical glia through Ascl1-mediated lineage reprogramming

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Loss of neural cells, mostly neurons or oligodendrocytes, is causally associated with many neurological disorders.

To supply the CNS with new functional neural cells, we reprogram glia by forced expression of transcription factors, previously shown to regulate cell fate during development. Here, we investigated the reprogramming potential of the pro-neural factor Ascl1 *in vivo* and tested whether post-translational modifications affected the reprogramming outcome. For this, we transduced proliferative glia with retroviruses encoding for Ascl1 wild-type or Ascl1SA6, a phospho-deficient variant generated by the mutation of six conserved serine-proline phosphorylation sites (Li et al., 2014) by virus injection in the early postnatal (P5) mouse cortex. In sharp contrast with their high efficacy of reprogramming cultured astrocytes into neurons, both Ascl1 variants failed to induce neurogenesis from glia *in vivo*. Instead, we observed a shift in the proportion of cells expressing the astroglial marker GFAP towards expression of the oligodendroglial lineage marker Sox10. Remarkably, co-expression of Bcl2 (Gascón et al., 2016) enabled conversion into neurons expressing DCX or NeuN. The reprogramming efficiency was higher with Ascl1SA6 and strikingly, a larger proportion of Ascl1SA6/Bcl2 induced neurons acquired a parvalbumin interneuron identity. Consistently, Ascl1SA6/Bcl2 reprogrammed cells had more mature functional electrophysiological properties and were capable of fast-spiking firing (see poster by Nicolás Marichal). Finally, our data indicates that Ascl1SA6/Bcl2 is also more efficient than Ascl1/Bcl2 for reprogramming cortical glia in the adult lesioned cortex. Together, our results indicate that Ascl1-induced fate decisions are fine-tuned by environmental factors and interference with its post-translational regulation, and this could be exploited to generate disease-specific cell types for CNS repair.

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T16-031E

Endothelin-1 signaling regulates the neural stem cell response to demyelination

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Demyelination occurs in a large variety of central nervous system (CNS) insults, pathologies, and neurodegenerative diseases, including Multiple Sclerosis (MS) and Charcot-Marie-Tooth disease. Current therapies for relapsing-remitting MS can be effective in reducing symptoms to an extent but have little impact on promoting remyelination. Parenchymal oligodendrocyte progenitor cells (OPCs) located throughout the CNS participate in endogenous remyelination of white matter lesions, migrating to the injury site and maturing into myelinating oligodendrocytes, albeit at low levels. An additional source of OPCs following CNS injury is neural stem cell (NSC)-derived OPCs generated in the subventricular zone (SVZ) of the lateral ventricles. Importantly, previous studies have found that these NSC-derived OPCs demonstrate greater propensity to differentiate into mature oligodendrocytes compared to parenchymal OPCs, generating thicker myelin sheaths. Therefore, promoting increased levels of NSC gliogenesis is a promising therapeutic strategy for demyelinating disorders like MS. However, NSC research has primarily focused on adult neurogenesis; thus, the molecular mechanisms behind adult NSC gliogenesis remain largely unknown. We investigated the role of the Endothelin-1 (ET-1) signaling pathway in the adult mammalian SVZ because of its known roles in glial physiology. We found that all NSC populations in the adult mouse SVZ express the ET-1 receptor, Ednrb. Ablation of Ednrb from NSCs reduced both

the number of activated and quiescent NSCs, indicating that ET-1 signaling is required for maintenance of NSCs in the healthy adult mouse. Following focal demyelination of the corpus callosum, SVZ NSCs upregulated expression of ET-1. Ablation of ET-1 reduced the percentages of proliferating NSCs and proliferating OPCs in the SVZ, suggesting that ET-1 plays a critical role in the SVZ proliferative response to injury. RNAseq of cultured primary NSCs and OPCs treated with ET-1 identified genes involved in stem cell maintenance, including Notch signaling, and OPC migration. Lastly, we confirmed that ET-1 and EDNRB expression are conserved in the adult human SVZ, indicating that this pathway may be a potential target for promoting SVZ-mediated cellular repair.

T16-032E

Phloretin enhances remyelination by stimulating OPC differentiation

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Failure of remyelination underlies the progressive nature of demyelinating diseases such as multiple sclerosis. Why endogenous repair mechanisms frequency fail in these disorders is poorly understood, however, there is now strong evidence that this is related to an overly inflammatory microenvironment combined with the intrinsic inability of oligodendrocyte precursor cells (OPCs) to differentiate into mature myelinating cells. Previously, we found that phloretin, a flavonoid abundantly present in apples and strawberries reduces neuroinflammation by driving macrophages towards an anti-inflammatory phenotype. Here, we show that phloretin also markedly stimulates remyelination in *ex vivo* and *in vivo* animals models. However, improved remyelination was attributed to a direct impact of phloretin on OPC maturation and occurred autonomously from alterations in microglia function and inflammation. Mechanistically, phloretin activated the fatty acid sensing nuclear receptor peroxisome proliferator-activated receptor gamma PPARy, thereby promoting the maturation of OPC. Altogether, our findings indicate that phloretin has pro-regenerative properties in CNS disorders, with potentially broad implications for the development of therapeutic strategies and dietary interventions.

T16-033E

Smoothened signalling during OPC differentiation

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Myelin regeneration or remyelination in the central nervous system (CNS) is an essential process that is activated

after demyelinating events that occur under various pathological conditions. This spontaneous response can be detected in animals after experimental lesions and in humans with multiple sclerosis, the most common demyelinating disease of the CNS. However, the repair process fails if it occurs at early steps of the disease while the disease progresses leading to the loss of the metabolic support normally provided by myelin to axons. This failure subsequently results in axon degeneration and irreversible neurological disabilities. Several compounds including Clobetasol, Gefitinib, Clemastine, Benztropine, Clotrimazole, and Miconazole were identified recently in repurposing phenotypical screenings based on their ability to promote MBP expression in primary cultures of oligodendrocyte progenitor cells (OPCs), in the Oli-neuM cell line, or Epiblast-derived OPCs (Cunniffe & Coles, 2021; Del Giovane & Ragnini-wilson, 2018). Subsequent validation studies have clarified that these molecules promote OPC differentiation by targeting specific receptor/pathways including muscarinic receptors (Benztropine, Clemastine), the glucocorticoid and smoothened (Smo) receptors (Clobetasol), or the accumulation of 8,9-unsaturated sterols (Benztropine, Clotrimazole, Miconazole). However, only Clemastine has reached the clinical trial phase (Cunniffe & Coles, 2021). It is therefore presently difficult to determine the impact that phenotypical screens will have on the clinical application of these remyelinating agents. Understanding the mechanism of action of promyelinating drugs identified after phenotypical screens remains the most challenging part of the preclinical drug discovery pipeline. However, it is clear that there will be a greater probability of success at the clinical level with a greater understanding of the biological basis of drug action in preclinical studies. Our group has isolated Clobetasol and Gefitinib in a phenotypical screen in the Oli-neuM cell line (Porcu et al., 2015). Furthermore, we have shown that Gefitinib promotes Oli-neuM differentiation until artificial axon engagement via an EGFR/PIP2/RxRγ pathway (Nocita et al., 2019). Our most recent data were focused on the clarification of the role of the Smo receptor during the last stages of OPC differentiation. To this end we used, in addition to Clobetasol, Smo agonists and antagonists as well as a gene silencing approach. Altogether, our data support the view that Smo receptor activation is required after myelin regulatory transcription factor (MyrF) expression, during the entire OPC differentiation process until axon engagement, via the activation of a non-canonical pathway.

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T16-034E

Secreted factors from activated antigen-specific CAR-Treg enhance oligodendrocyte differentiation and *ex vivo* remyelination

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One of the greatest unmet clinical needs in demyelinating diseases such as Multiple Sclerosis (MS) is a lack of therapies that actively enhance remyelination in the central nervous system (CNS). Uncovering the mechanisms that govern remyelination and developing novel remyelinating treatments may prove highly beneficial to patients' quality of life. Oligodendrocytes, the myelinating cells of the CNS, play a central role in remyelination and originate from oligodendrocyte progenitor cells (OPCs). We have recently shown that regulatory T cells (Treg) are important mediators of oligodendrocyte differentiation and remyelination.* Chimeric antigen receptor (CAR)-Treg are being developed as cellular therapies based on immune-modulating functions and potential to induce tolerance. Based on our findings with conventional Treg, we hypothesise that CAR-Treg also positively influence myelin regeneration responses. This study aims to determine the effect of secreted factors from activated, antigen-specific CAR-Treg on key steps in the process of myelin regeneration: oligodendrocyte proliferation and differentiation.

To investigate the potential of CAR-Treg to enhance oligodendrocyte differentiation, murine OPC cultures were treated with conditioned media from CAR-Treg activated through the CAR by their specific antigen, conditioned media from Treg with control constructs or media controls for four days. Murine brain slices were demyelinated using lysolecithin and allowed to remyelinate for one week while treated with CAR-Treg-conditioned media or appropriate controls.

Secreted factors from CAR-Treg or polyclonally activated Treg both enhanced oligodendrocyte differentiation compared to media controls to a similar degree. There was no effect on OPC purity or proliferation observed in any treatment. Furthermore, factors secreted by CAR-Treg after antigen specific stimulation through the CAR enhanced remyelination *ex vivo* to a higher degree than polyclonal activation through the TCR.

These studies show that antigen-specific CAR-Treg demonstrate capacity to enhance oligodendrocyte differentiation and remyelination, highlighting additional beneficial mechanisms of this potential treatment for MS patients, beyond immunomodulation. The regenerative potential of antigen-specific CAR-Treg will be further studied in *in vivo* murine models of remyelination.

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T16-035E

Membrane targeting peptide: new drug to target LINGO-1, p75, TROY and AMIGO3 platform to reverse remyelination failure in MS

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Multiple sclerosis (MS) is a neuroinflammatory and demyelinating disease of the central nervous system. Treatments target the inflammation part of the disease with immunosuppressing or immunomodulating treatments to decrease the immune system activation against myelin antigens. However, these treatments have important side effects. Thus, there is urgent need to develop new therapeutic approaches to treat the disease and enhance patients' everyday life. LINGO1, p75 TROY and AMIGO3, in association with NogoR1 are designated targets to treat MS. As many other guiding molecules such as semaphoring 3A, NogoA is over expressed in MS patients' lesions and contributes to the remyelination failure. NogoA binds to its receptors inducing RhoA pathway activation and inhibit oligodendrocytes migration and differentiation. Blocking the signalling platform of NogoA has been proposed to enhance the remyelination process (Mi et al., 2007, Jepson et al., 2012). Based on membrane targeting peptide (MTP) technology previously developed (Pham-Van Binamé, 20019), we here designed antagonist peptides for each target: MTP-LINGO1, MTP-P75, MTP-TROY, and MTP-AMIGO3. MTPs are mimicking the transmembrane segment of the target protein and are intended to antagonize receptor mediated-signaling by blocking dimerization. We first conducted an *in silico* analysis using PREDDIMER software to characterize the potential of transmembrane domain interactions for these targets. A second approach with PISA identified the amino acid residues involved in the identified interactions. From these data we developed a Bioluminescence energy transfer technology BRET assay to monitor full length protein association. We confirmed the results of homo- and hetero-associations by co-immunoprecipitation assays. The systematic analysis of all four peptides identified the best interacting peptides with targeted TM domains. Hence; to study the functional blocking activity of selected peptides, we performed an automatized migration in vitro assay (Xcelligence) with Oli-neu cell repelled by NogoA as an inhibitor of migration. Overall, this study identified new interfering peptides antagonizing the NogoA inhibitory activity to be challenged in relevant preclinical models of the disease.

T16-036E

Plagl1 is required to sustain murine Müller glial cell quiescence and retinal homeostasis

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Müller glia are essential for retinal homeostasis, maintaining structural integrity, physiological function, and

providing neurotrophic support. Müller glia also have an added role as stem cell-like cells in fish and frogs, but this regenerative property is lost in mammals. We previously showed that the imprinted gene *Plagl1*, encoding a zinc finger transcription factor, is expressed in murine Müller glia. Strikingly, a comparison to published scRNA-seq datasets revealed that Plagl1 is upregulated in the first few hours post-injury, but then rapidly downregulated. To test Plagl1 function in the perinatal retina, we examined *Plagl1*$^{+/-pat}$ null mutants, identifying defects in retinal architecture and visual signal processing at perinatal stages. Moreover, *Plagl1*$^{+/-pat}$ Müller glia become gliotic and proliferate ectopically in the postnatal retina. Birthdating and lineage tracing of these proliferating Müller glia revealed that they give rise to new Müller glia, but also some rod photoreceptors and amacrine cells. Transcriptomic and molecular profiling of perinatal *Plagl1*$^{+/-pat}$ retinas revealed similarities with other retinal degenerative models, including upregulation of pro-gliogenic and pro-proliferative pathways, including ERK, Notch and Hippo pathways. Strikingly, the *Plagl1*$^{+/-pat}$ transcriptome closely resembles the transcriptome of Müller glia early post-injury. *Plagl1* is thus an essential component of the transcriptional regulatory networks that retain mammalian Müller glia in quiescence and sustain retinal homeostasis.

T16-037E

Potential role of G protein-coupled receptor signalling in glia-to-neuron conversion in the postnatal mouse cerebral cortex

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Glial cells (i.e., astrocytes and oligodendrocyte precursors) residing in the brain can be reprogrammed into induced neurons by forced expression of neurogenic transcription factors (TFs) (Heinrich et al. 2014, Gascón et al, 2016). We have shown that populations of proliferative glial cells of the mouse postnatal cortex can be targeted by retroviruses (RVs) encoding for reprogramming factors, e.g. proneural genes such as Neurog2 or Ascl1 in combination with Bcl2. Forced expression of Neurog2 and Bcl2 yields in high glia-to-neuron conversion efficiencies. Importantly, the proliferative state of iNs prior to reprogramming is confirmed with EdU incorporation. Nevertheless, glia-derived iNs remain immature in terms of morphology and functional properties. Thus, one major goal aims at improving iN maturation. Functional maturation of iNs has been improved by the co-expression of neurogenic TFs with excitatory DREADDs (designer receptor exclusively activated by designer drugs), the receptor hM3Dq, which, after its activation by clozapine-N-oxide (CNO) activates a Gq-protein coupled receptors (GPCRs) signalling pathway resulting in increased excitability of iNs. In this project, we aim at studying if the activation of endogenous GPCRs systems affects the maturation of iNs. Given the well-known role of dopamine in the maturation and modulation of newly generated cortical neurons during development (Stanwood et al., 2005, Wang et al., 2009; Berlanga et al., 2011), here we aim at inducing glia-to-neuron conversion in the postnatal cerebral cortex of P5 mice in which dopaminergic neurons express the receptor hM3Dq. In this model, we perform immunohistochemical, morphological and functional analyses to reveal the degree of iN maturation upon dopaminergic activation. In addition, we are currently performing *in situ* hybridization assays (i.e. RNAscope) in combination with immunohistochemistry to uncover whether iNs express dopamine receptors. Altogether, these experiments will allow us to assess the effect of dopamine signalling on the maturation and integration of glia-derived iNs.

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T17 | Transmitter receptors, ion channels and gap junctions

T17-001A

ADAM23 and LGI proteins govern the functional organisation of K_v1 channels in myelinated axons

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Excitability of cellular membranes is largely governed by the distribution, density and biophysical properties of expressed ion channels. In myelinated neurones, these are regulated through interactions between the axon and the neighbouring glial cell. Here, we focus on a particular family of ion channels – Shaker-type voltage-gated potassium channels (K_v1). These are expressed predominantly in axons, in particular at the axon initial segment (AIS) and the juxtaparanode (JXP), a region of myelinated axons flanking the node of Ranvier. Here, they are thought to regulate the membrane potential, protecting the axon from hyper-excitability. Consequently, pathology related to K_v1 channels has been reported in epilepsy, neuromyotonia, ataxia and neuropathic pain – all characterised by disrupted neuronal signalling. Moreover, K_v1 dissociation from the JXP is an early sign of axonal demyelination and often proceeds worsening of symptoms in demyelinating conditions. It is therefore essential that we understand the molecular processes that govern the functional organisation of K_v1 at the JXP.

Previous research suggested that the accumulation of K_v1 channels at the JXP is dependent on their association with Caspr2 and Tag-1, as well as the adaptor protein 4.1B. However, later studies into the mechanisms of the JXP organisation identified gaps in the previously accepted model and suggested that additional proteins are involved in this process. Recent studies pointed towards the association of K_v1 with the ADAM proteins; ADAM11, ADAM22 and ADAM23. These are known to interact with LGI proteins in a receptor-ligand fashion. The LGI family consists of four secreted proteins, all expressed in the nervous system. Preliminary data from our lab show that ADAM23 is present at the JXP where it interacts with LGI2 and LGI3.

We employed several genetic mouse models to investigate the importance of these interactions in clustering and maintenance of K_v1 complexes at the JXP as well as their regeneration after axonal injury. These *in-vivo* experiments were complemented by *in-vitro* investigation to characterize the nature of the molecular interactions that take place within the JXP complex. Our data indicate that the expression of ADAM23 in the axonal membrane within the JXP domain, and its interaction with LGI2 and LGI3, are crucial for the functional organisation of K_v1 complexes, not only during development but also in maintenance and axonal regeneration. Furthermore, we investigated the involvement of ADAM23 in transport of K_v1 channels to the axon and now propose a mechanism through which LGI-ADAM23 interactions might affect K_v1 accumulation at the JXP. Whether these interactions are also of importance for K_v1 localisation at the AIS is the subject of further research.

T17-002A

Unconventional role of TRPV4 in astrocytes swelling

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More than any other neuronal or glial cell, astrocytes experience severe osmotic challenges and cell volume variations, with a marked tendency for cellular swelling due to the massive expression of Aquaporin-4 (AQP4). AQP4 is functionally coupled with the calcium-channel Transient-Receptor Potential Vanilloid member-4 (TRPV4) in water transport dynamics. However, the molecular events underlying AQP4 and TRPV4 interplay remain a matter of debate. The present work aims to investigate the selective role of TRPV4 in regulating water membrane permeability. For such purpose, HEK293 cells natively lacking of AQP4 were transfected with TRPV4-eGFP and used in water transport measurements performed with Calcein-AM quenching assay to determine the rate of cellular swelling under hypotonic conditions in the presence of TRPV4 chemical modulators. Results show that the chemical activation of TRPV4 with 4αPDD leads to a three-fold increase in the rate of water transport in the swelling phase compared to activated but non-transfected controls, while a three-times slower kinetic is observed in HEK-TRPV4 when treated with TRPV4-specific antagonist RN-1734 compared to activated cells. These findings support the involvement of the cation channel in the regulation of water membrane permeability in an AQP4-independent manner, giving rise to the question of which elicited mechanism can cause the founded effect. Volume Regulated Anion Channel (VRAC) is one of the major players involved in cell volume regulation mechanisms. Due to its relatively large pore diameter ranging from 6 to 8 Å, it has been proposed that VRAC can contribute to the exchange of water itself. However, such a permeation has never been proved. Therefore, assuming that VRAC could be permeated by water molecules, we investigated whether the activation of TRPV4 could lead to the establishment of an interplay TRPV4-VRAC where the former, by acting on the latter, enhances its water permeability. Preliminary results in HEK show that TRPV4 over-expression does not influence the expression levels of VRAC. On the other hand, water measurements performed in HEK treated with siRNA directed against the functional VRAC subunit LRRC8A and in LRRC8A-KO HCT116 cells, both over-expressing TRPV4, prove that the activation of TRPV4 can increase the speed of water transport by three and two-fold, respectively, regardless of the presence or absence of a functional VRAC channel. Altogether these findings demonstrate that TRPV4 facilitates osmotic flows in hypotonicity-induced swelling in AQP4 and VRAC-independent ways. Future studies will be focused on exploring the molecular pathways underlying the TRPV4 effect in promoting a faster plasma membrane water permeability.

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T17-003A

TRPV4-induced Ca^{2+}influx is required for microglial motility

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BACKGROUND: The dynamism of microglial branches allows for the continuous surveillance of the brain parenchyma, a process that is essential for both brain development and instant responses to brain damage. Branch motility is tightly connected to cytoskeletal rearrangement and strictly regulated by intracellular Ca^{2+}. Although chemical extracellular cues (e.g. ATP) are potent modulators of intracellular Ca^{2+}, the activity of chemoreceptors does not affect microglia process movement. Hence, the identity of the Ca^{2+}-permeable channel(s) directly fueling branch motility remains elusive. Transient receptor potential vanilloid 4 (TRPV4) has been shown to be functionally expressed in lamellipodia, soma and extended processes. Furthermore, the channel is known for its important role in cellular motility and cytoskeletal systems making it a potential key player in process movement. In this study, we aim to explore the role of the mechanosensitive cation channel TRPV4 in the control of branch motility in microglia. **RESULTS:** Using live intracellular Ca^{2+} imaging, here we show that TRPV4 is functionally expressed in freshly isolated microglial cells. We demonstrated that genetic ablation of TRPV4 decreases the growth of ramified processes in microglial cells. Moreover, acute inhibition of TRPV4 activity in primary microglia halts tubulin dynamics, induces a shift towards an amoeboid morphology and hinders microglial persistent random walk. Time-lapse multiphoton microscopy on acute brain slices showed that genetic ablation or pharmacological inhibition of TRPV4 decreases the area surveyed by microglial processes, while TRPV4 knockout microglia display unaltered process extension towards lesion. **CONCLUSION:** We identify TRPV4 as a player in the regulation of microglial motility.

T17-004A

Traumatic brain injury induces a heterogeneous response in Cx43, affecting astrocytic coupling

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Astrocytes couple into networks of hundreds of cells. Impaired astrocyte coupling is associated with epilepsy, but there is no consensus on whether reduced coupling promotes or counteracts abnormal neuronal activity, which

precedes seizures. In acquired epilepsy, which is initiated by a neurological insult such as traumatic brain injury (TBI), many studies have demonstrated dysfunction of gap junctions (GJs) and decrease of Connexin43 (Cx43), the major protein forming gap junctions. Other studies suggested an increased Cx43 expression correlated to seizures. An integrated view that accounts for both findings is needed to reveal how Cx43 changes in regulation modulates epilepsy after TBI.

In a new model of of TBI we found heterogeneity in Cx43 regulation: a subgroup of astrocytes lost Cx43 expression, while total Cx43 was increased in the cortex. We evaluated connectivity by filling single astrocytes with biocytin, which spreads through GJ to neighboring cells. We observed a decrease of coupling between astrocytes with reduced Cx43 and the rest of the astrocytic network. Furthermore, Cx43 Ser368 phosphorylation was increased after TBI, a post-translational modification associated with decreased GJ function. Therefore, we concluded that a subset of astrocytes presents a disrupted connectivity.

On the other hand, we also found an increase in non-gap junctional Cx43 through solubility assays. Cx43 can form hemichannels that might open under pathological conditions releasing excitatory molecules and contribute to the brain hyperexcitability (fig1). Biotinylation assays and EtBr uptake experiments indicated an increase of Cx43 hemichannel expression and function after TBI.

These results suggest misregulation of Cx43 after TBI causes a decrease in astrocytic coupling and a simultaneous increase in hemichannel activity. Therefore, both a decrease in junctional Cx43 and astrocytic coupling can happen concomitantly to an increase in hemichannel Cx43 expression and function after TBI, both favoring neuronal hyperexcitability (fig2).

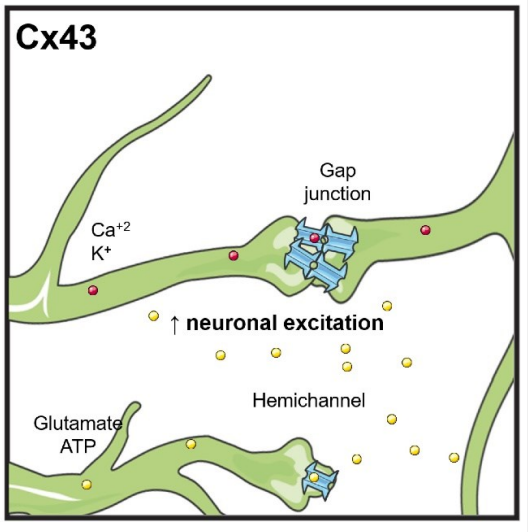

Figure 1. Cx43 can form gap junctions or hemichannels
Cx43 is the major protein forming gap junctions in astrocytes, allowing astrocytes cytoplasm coupling. Cx43 can also form hemichannels that under pathological conditions might open, releasing excitatory molecules such as glutamate

or ATP.

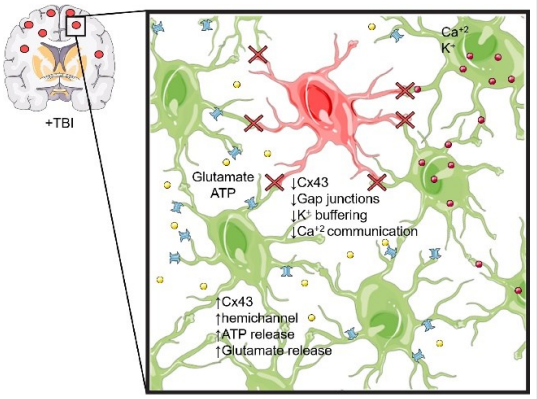

Figure 2. Cx43 function is disrupted in astrocytes after TBI
Model summarizing the Cx43 changes following TBI. After TBI, gap junctions formed by Cx43 are decreased, impairing the astrocytic coupling. On the other hand, Cx43 is increased forming hemichannels that favor the release of excitatory substances.

T17-005A

Mapping Cx43 surface to prevent hemichannel coupling

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Astrocytes show highly synchronized activity during epilepsy- Moreover, the synchronization of astrocytes precedes the synchronization of neurons, therefore astrocytes are proposed to be the source for neuronal synchronization (Héja, 2014; Kardos et al., 2016). This process is presumed to involve Cx43 connexin (Cx) channels that are the dominant Cx subtype on astroglial cells. In contrast, Cx36 is the major subtype on neurons. Deeper understanding and utilization of Cx43 however, is greatly reduced by the lack of an effective, Cx43 specific inhibitor. To overcome this situation, we applied molecular dynamics (MD) methods on Cx43 and Cx36 models to search for potential surface sites suitable for Cx43 specific inhibition of hemichannel (HC) coupling.

Three different approaches have been addressed using recently determined 3D structures of Cx proteins. 1) Docking of frequently used peptide inhibitors that are thought to be Cx43-specific. 2) Identification of stabilization centers (residue pairs, responsible for the thermal stability of proteins) to find potential target sites to inhibit HC

coupling. 3) Examination of disulfide bonds in gap junctions (GJs), based on the observation that extracellular loops are held together by an extensive disulfide bonding system in each chain.

Based on the first 3D structure of a Cx26 GJ (Maeda et al., 2009), we developed homology models of Cx43 and Cx36 HC. Inhibitory peptides (GAP26, P5 or P180–195, GAP27, Peptide5) and the moderately Cx36-specific inhibitor quinine were docked into the Cx43 and Cx36 HC models, but no subtype specificity was observed, even after a 50 ns all-atom MD simulation in explicit lipid environment. (Simon et al., 2020). However, we found that stabilization centers appear at the extracellular region of HC chains, providing important connections in the full GJ. Consequently, we modelled the full Cx43 GJ and Cx36 GJ, where a completely different set of stabilization centers could be identified, highlighting targetable residues (Fig. 1.) Recently determined GJ structures revealed disulfide bonds connected to these HC/HC interface stabilization centers, therefore we investigated these in MD runs.

We embedded the full Cx43 GJ in two membranes, mimicking two cell membranes and performed 100 ns all-atom MD simulations in explicit lipid environment with and without closing disulfide bonds, to investigate the effect of potential disulfide exchange. Our simulations revealed that disulfide-related stabilization centers delineate a tetrapeptide that can potentially target the Cx43 HC surface. In summary, the prevention of GJ formation at the HC/HC interface by a stabilization center-derived small peptide provides a reliable strategy for subtype-specific, effective inhibitor design.

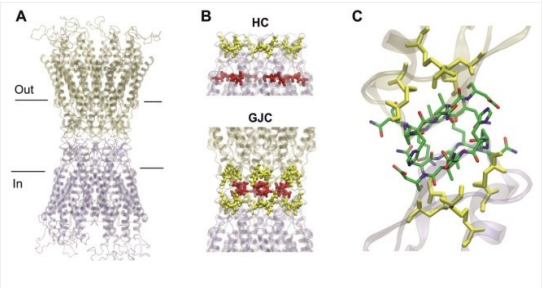

Fig.1.
Critical localization of stabilization centers and disulfide bonds in GJC. (A) 3D structure of the Cx43 GJC model. The two HCs are colored brown (chains G-L) and purple (chains A-F). (B) Stabilization centers are easily distinguishable in Cx43 HC and GJC models. Stabilization centers in HC appear at the membrane surface, while those in the full GJC are at the HC/HC interface. Stabilization centers are shown in red, Cys residues forming disulfide bonds are shown in yellow. (C) The HC/HC interface (green) is surrounded and potentially positioned by 3 disulfide bonds (yellow) in each chain.

T17-006A

$GABA_B$ receptor downstream mechanisms in OPCs and its role in myelination and remyelination

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Oligodendrocyte (OL) differentiation from oligodendrocyte progenitor cells (OPCs) is responsible for myelin formation in the central nervous system (CNS) during physiological processes, such as development, and after demyelinating lesions, as in multiple sclerosis (MS). These processes are regulated by neuron-OL communication through the action of specific molecules such as neurotransmitters, including γ-aminobutyric acid (GABA). Our previous work has demonstrated that *in vitro* activation of oligodendroglial $GABA_B$ receptors ($GABA_BR$) enhances OPC differentiation and the expression of the myelin related proteins. In the present study, we aim to identify the downstream mechanisms that mediate $GABA_BR$ response in OPCs and to evaluate the importance of *in vivo* systemic $GABA_BR$ activation with its agonist baclofen (Bac) for myelination during development and for remyelination after lesions. First, we analyzed by RT-qPCR changes in gene expression in isolated OPCs and we detected an upregulation in *myrf*, *mbp* and *plp* expression promoted by Bac treatment. In addition, we evaluated in cultured OPCs Bac-induced changes in the phosphorylation state of molecules involved in OPC survival and differentiation, such as FAK, Akt, Src and CREB by immunoblotting, and we observed that Bac caused significant alterations in the phosphorylation of these molecules. In order to deepen in the molecular mechanisms triggered by $GABA_BR$ activation in OPCs and to establish the intracellular signaling pathways involved, we also conducted a transcriptomic analysis through RNA sequencing in cultured OPCs following 4-hour Bac treatment. The obtained data provide relevant information on $GABA_BR$-mediated signaling in OPCs *in vitro*. On the other hand, we assessed the impact of *in vivo* Bac administration on OPC differentiation and myelin formation during rat development and after demyelinating lesions in mice spinal cord, by confocal and transmission electron microscopy. Focusing first on the analysis of myelination during development, we administered Bac (4 mg/kg/day) to developing rats and we observed changes in different white and grey matter areas of the brain. Moreover, we treated adult mice with Bac (8 mg/kg/day) following lysolecithin-induced spinal cord demyelination and we analyzed the differences in OPC differentiation and the remyelination rate within the lesions. Finally, we detected changes in the level of axonal damage and inflammation biomarkers in plasma samples of MS patients clinically treated with Bac as an antispasmodic therapy. Overall, this work defines $GABA_BR$ downstream mechanisms in OPCs and point out to this receptor as a potential regulator of myelination.

T17-007A

Cholesterol-dependent mobility and activity of Pannexin1 channel

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Pannexin1 (Panx1) forms large conductance channels permeable to ATP. In the central nervous system (CNS)

Panx1 is expressed in both glia and neurons and plays a pivotal role in numerous pathological conditions such as epilepsy, migraine, inflammation, and ischemia. Cholesterol in the membrane is a major determinant of protein mobility, thereby affecting the proper distribution and function of receptors and ion channels. Defects in cholesterol metabolism are increasingly associated with neurological diseases including Alzheimer's disease, Huntington's disease, and autism. In order to understand factors that regulate the distribution and function of Panx1 in the cell membrane, we have used Fluorescence Recovery After Photobleaching (FRAP) to evaluate the mobility of Green Fluorescent Protein (GFP)-tagged Panx1 and electrophysiological and dye uptake to assess channel function. In initial studies, we examined the effects of cholesterol on Panx1 cell surface dynamics. Our experiments indicate lower lateral diffusion parameters of Panx1 in cholesterol-depleted cell plasma membrane caused by treatment with cyclodextrins (methyl-β-cyclodextrin and others) and by metabolic inhibition (Lovastatin). The fluorescence and electrophysiology measurements performed in cholesterol-depleted cell plasma membranes in hPanx1 transfected cells and in wild-type astrocytes showed higher dye uptake and greater channel activity compared to Panx1-null cells. Moreover, quantification of the amount of ATP released from wild-type astrocytes and cholesterol-depleted hPanx1 N2a transfected cells indicated that the absence of Panx1 prevented ATP release from these cells. Analysis of the distribution of Panx1 and P2X7 receptors using super-resolution microscopy revealed a highly significant increase overlap induced by cyclodextrin treatment. We conclude from these studies that cholesterol modulates Panx1 mobility through effects on membrane organization with deletion increasing interaction with P2X7 receptors and enhanced channel activity, and manipulation of cholesterol levels may therefore offer a novel treatment by which to regulate activation of Panx1 channels, thereby reducing deleterious consequences of ATP release in diverse neurological syndromes.

T17-008A

Astrocyte miRNA transfer occurs via connexin-43 gap junctions

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Glia balance ions and metabolites throughout the central nervous system via gap junctions[1]. However, gap junctions have the capacity to flux molecules over 1kD in size, an order of magnitude larger than most metabolites used within the central nervous system. In other systems, gap junctions serve another critical function – they redistribute miRNAs. Of all connexins, connexin 43 (cx43) is particularly miRNA permeable[2]; cx43 is also the connexin astrocytes use in the majority of their gap junctions[3]. The capacity for miRNA redistribution through astrocyte gap junctions, however, is unexplored.

Here, we use primary cortical immunopanned astrocytes[4] from wild-type Sprague Dawley rats and astrocyte-directed conditional cx43 knockout marker mice (GFAP-cre-ER^{T2} x cx43$^{fl/fl}$ x ROSA-lacz$^{fl/fl}$; cx43 cKO) to examine the capacity for astrocytes to transfer RNAs through gap junctions. Cortical rat astrocyte cultures (post-isolation day 10-14, maintained in serum-free culture conditions) were cut-loaded with fluorescein-tagged miRNA[2] (miR-F; 20 μM, in media) for 10 minutes, then fixed in 4% PFA. Without the gap-junction inhibitor carbenoxolone (CBX), miR-F transfer occurs between astrocytes as far as 300um from the cut-load site; this transfer no longer occurs when astrocytes are pre-incubated in 100 μM CBX for 10 minutes ($n = 4$ isolations, repeated 8 times per isolation; $p < 0.001$). To determine whether cx43 was responsible for miRNA transfer, cortical astrocyte cultures from cx43 cKO

mice (post-isolation day 16-20) were exposed to 4-hydroxytamoxifen (4-HT; 1 μM, in media) for 3 days to induce KO, then cut-loaded with miR-F (20 μM, in media) for 10 minutes followed by fixation in 4% PFA. Mouse cx43 cKO astrocytes transferred miR-F up to 140 μm from the cut-load site, and this transfer was halted by 4-HT exposure (n = 2 isolations, repeated 8 times per isolation; $p < 0.001$).Understanding astrocyte capacity for miRNA redistribution is important, as these small, non-coding RNAs post-transcriptionally regulate gene expression. Transferring miRNAs through gap junctions would provide cells with a rapid, energetically conservative mechanism to alter the expression profiles of each cell within their network. To better understand the dynamics of astrocyte network plasticity and how plasticity can be regulated, we need to understand the suite of molecules astrocyte networks are capable of transferring through connexins. This knowledge would alter the way we understand astrocyte networks communicate throughout the brain and illuminate a novel avenue for therapeutic intervention by manipulating the transcriptomic profile of populations of interconnected astrocytes.

Acknowledgement

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T18 | Trophic factors

No abstracts have been submitted.

T19 | Tumours

T19-001C

TAMEP are brain tumor parenchymal cells controlling neoplastic angiogenesis and progression

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Aggressive brain tumors like glioblastoma depend on support by their local environment and subsets of tumor-parenchymal cells may promote specific phases of disease-progression. We investigated the glioblastoma microenvironment with transgenic lineage-tracing models, intravital imaging, single-cell transcriptomics, immunofluorescence analysis as well as histopathology and characterized a previously unacknowledged population of tumor-associated cells with a myeloid-like expression profile (TAMEP) that transiently appeared during glioblastoma growth. TAMEP of mice and humans were identified with specific markers. Notably, TAMEP did not derive from microglia or peripheral monocytes but were generated by a fraction of CNS-resident, SOX2-positive progenitors (Fig. 1). Abrogation of this progenitor cell-population, by conditional *Sox*2-knockout, drastically reduced glioblastoma-vascularization and -size. Hence, TAMEP emerge as a tumor-parenchymal component with strong impact on glioblastoma progression.

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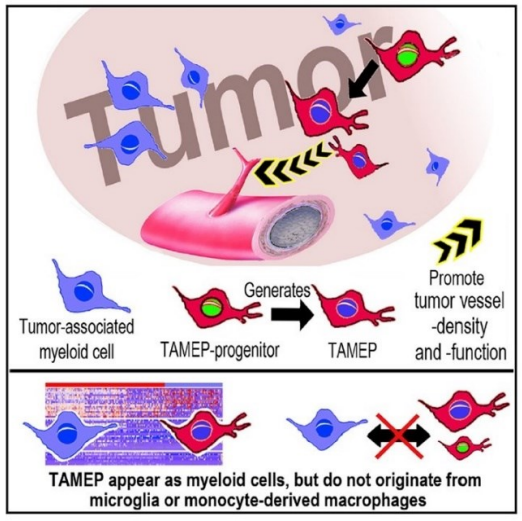

Figure 1
Graphical Abstract

T19-002C

Functional impact of *Tcf12* inactivation in glioma cells of origin and gliomagenesis

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Diffuse gliomas are frequent primary brain tumors of the adult, representing most of the malignant cases. Diffuse gliomas can be divided into three general types: astrocytomas, oligodendrogliomas and glioblastomas (GBM). Glioblastomas are the most aggressive type of gliomas, whereas astrocytomas and oligodendrogliomas are considered less aggressive; nonetheless they may recur as glioblastomas. Neural stem and progenitor cells (NSCs and NPCs) and oligodendrocyte precursor cells (OPCs) are the major candidates for the cells from which gliomas originate and are driven. Despite recent advances, the underlying mechanisms of the initiation and development of gliomas are far from fully elucidated, thus limiting currently used treatments to conventional radio- and chemotherapies.

Previous work done by our team identified, for the first time, heterozygous mutations in the gene of the bHLH transcription factor *TCF12* in oligodendrogliomas. Most of these mutations were inactivating and were associated with a more aggressive tumor phenotype. *TCF12* is implicated in several human cancers, acting either as an oncogene or as a tumor suppressor. In the case of gliomas, mining recent data from public resources, showed us that *TCF12* mutations were not exclusive to oligodendrogliomas, but were present in all gliomas, and that higher *TCF12* expression was associated with a better prognosis in patients. This study aims to elucidate the functional impact of *TCF12* mutation in glioma cells of origin and its role in gliomagenesis.

Tcf12 expression had been reported in embryonic and postnatal neural progenitors and glial cells in the mouse. We

validated that TCF12is expressed in murine OPCs and oligodendrocytes at different postnatal stages. By performing ChIP-Seq experiments, we observed that TCF12 occupies active promoter regions (marked by H3K4me3 and H3K27Ac) in murine NPCs and oligodendroglial cells (OPCs and oligodendrocytes), with the occupancy being higher in the latter. Moreover, we did observe that TCF12 binding peaks were shared with other transcription factors implicated in oligodendroglial lineage (OLIG2 and ASCL1). To address the consequences of TCF12 inactivation on OPCs, we generated and used a conditional and inducible knockout mouse model. Our results, from histological and transcriptomic analyses, imply a role of TCF12 in OPC proliferation and oligodendrocyte differentiation; however with the effects if its inactivation being transient. To study directly the role of *Tcf12* in gliomagenesis, we employed an immuno-competent GBM mouse model, in which *Tcf12* was highly expressed in tumor cells. Current studies focus on analyzing transcriptomic and histological data from tumors of this mouse model and comparing with data from human gliomas.

Acknowledgement

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T19-003C

Combined treatments with the dualsteric agonist N-8-Iper plus chemotherapy drugs affect drug resistance in glioblastoma cancer stem cells

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Glioblastoma multiforme (GBM), the most aggressive brain tumor in human, is characterized by heterogeneous cell populations, including Glioblastoma Stem Cells (GSCs) that share some similarities with Neural Stem Cells. Nowdays, one of the greatest challenges in the treatment of GBM is the multidrug resistance (MDR), a complex and multifactorial phenomenon involving a variety of mechanisms that make tumor cells resistant to different therapeutic treatments. In GBM, MDR has been observed especially in GSCs respect to non stem-like GBM cells. Our previous studies demonstrated that the selective stimulation of M2 muscarinic receptor subtypes by the orthosteric agonist Arecaidine Propargyl Ester (APE) decreased cell proliferation and survival, causing an arrest of the cell cycle both in GBM cell lines and in GSCs. In addition to the orthosteric binding site, muscarinic receptors also have allosteric binding sites. The different muscarinic subtypes show a high degree of sequence homology in the orthosteric binding site, which makes orthosteric ligands not always selective. They often lead to therapeutic ineffectiveness or numerous side effects due to the high dose required. For this reason, a new approach in drug design has been developed; the new synthetic compounds are called 'dualsteric ligands'. These molecules are hybrid derivates, containing the substructures of both orthosteric and allosteric ligands bridges by a spacer chain. N-8-Iper, is the most effective dualsteric agonist for the M2 receptor tested in our lab. Our study demonstrated that N-8-Iper was able to reduce GSC proliferation in the comparable manner of APE, but N-8-Iper showed a significant ability to reduce cell proliferation also at lower doses. The aim of the present study was to analyze the ability of the

M2 dualsteric agonist N-8-Iper to counteract GSCs drug resistance. By using sub-toxic concentrations of N8-Iper and of the single chemotherapy drugs, we found that only M2 agonist low doses combined to doxorubicin or cisplatin or temozolomide, are significantly able to counteract cell growth in different GSC cell lines (GB7 and G166 lines). The analysis of ABC efflux pumps expression levels following exposure to high and low doses of N-8-Iper, enlightened the ability of M2 receptor to significantly driving their downregulation. Our data demonstrated the ability of this novel M2 agonist to counteract drug resistance in GSCs at least partially through downregulating ABC drug efflux pumps expression. The combined effects of low doses of conventional chemotherapy drugs and M2 agonist may thus represent a novel promising pharmacological approach to impair the GSCs-drug resistance in GBM therapy.

T19-004C

Identification of CRYAB+ KCNN3+ SOX9+ astro-like cells and EGFR+ PDGFRA+ OLIG1+ oligo-like cells in diffuse low-grade gliomas and implication of Notch1 signalling in their genesis

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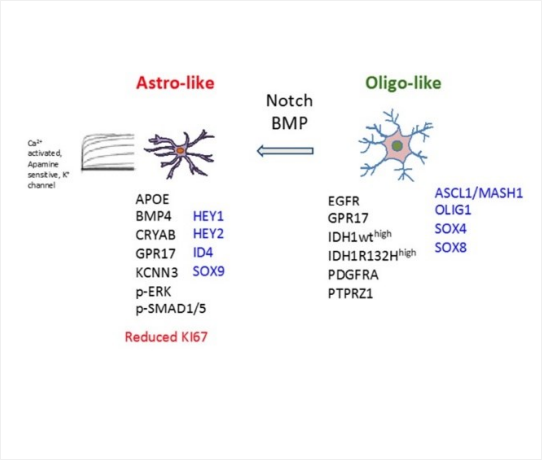
IDH1-mutated gliomas are slow growing brain tumours, which progress into high-grade gliomas. They present intra-tumoural cell heterogeneity, but no good markers are available to distinguish the different cell subtypes. The molecular mechanisms underlying the formation of this cell diversity is also ill defined. Here we report that the SOX9 and OLIG1 transcription factors, which specifically label astrocytes and oligodendrocytes in the normal brain, reveal the presence of two largely non-overlapping tumoural populations in IDH1-mutated oligodendrogliomas and astrocytomas. Astro-like SOX9+ cells additionally stain for APOE, CRYAB, ID4, KCNN3, while oligo-like OLIG1+ cells stain for ASCL1, EGFR, IDH1, PDGFRA, PTPRZ1, SOX4, and SOX8. GPR17, an oligodendrocytic marker, was expressed by both cells. These two sub-populations appear to have distinct BMP, NOTCH1, and MAPK active pathways as stainings for BMP4, HEY1, HEY2, p-SMAD1/5 and p-ERK were higher in SOX9+ cells. We used primary cultures and a new cell line to explore the influence of NOTCH1 activation and BMP treatment on low-grade glioma cell phenotype. This revealed that NOTCH1 globally reduced oligodendrocytic markers and IDH1 expression while upregulating APOE, CRYAB, HEY1/2 and an electrophysiologically Ca2+-activated apamin-sensitive K+ channel (KCNN3/SK3). This was accompanied by reduction in proliferation. Similar effects of NOTCH1 activation were observed in non-tumoural human oligodendrocytic cells, which additionally induced strong SOX9 expression. BMP treatment reduced OLIG1/2 expression and strongly upregulated CRYAB and NOGGIN, a negative regulator of BMP. The presence of astro-like SOX9+ and oligo-like OLIG1+ cells in diffuse

low-grade gliomas raise new questions about their role in the pathology (see the Graphical Abstract).



Graphical Abstract
Main figure of the work

T19-005C

Metabolic modules distinguishing glioblastoma cells in distinct functioning states identified from single cell analyses

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Glioblastoma cell ability to adapt their functioning to microenvironment changes and therapies, is one of the sources of the extensive intra-tumor heterogeneity characteristic of this devastating malignant brain tumor. Metabolism being at the core of cell functioning, we sought for metabolic pathways underlying cell functioning states in the complex setting of fully-grown patient tumors, as encountered at the time of diagnosis.
We combined analyses of independent single-cell transcriptomes from surgical resections, and experimental manipulation of in vivo human glioblastoma models.
Unsupervised analyses revealed that information dispersed throughout the cell transcriptome encodes each tumor identity. In contrast, data reduction based on experimentally-defined molecular signatures unmasked information on cell functioning state potentials. Modeling the expression network of the metabolic pathways distinguishing cells with high tumorigenic potential highlighted the very long chain polyunsaturated fatty acid synthesis pathway at the core of the network. Expression of its most downstream enzymatic component, ELOVL2, was shown to be required for cell tumorigenicity in vivo. Current analyses support further the relevance of our approach to identify metabolic pathways and enzymes characterizing additional functioning states essential for glioblastoma growth.
Our results demonstrate that single-cell transcriptomes from patients' glioblastoma can be harnessed to obtain an integrated view of metabolic pathways at play within patient tumors, and to identify targets of high therapeutic relevance.

T19-006D

Ubiquitin-proteasome system and mitochondrial respiration alterations andoxidative stress induction in human glioblastoma cells: role of the M2 muscarinicreceptors

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Glioblastoma Multiforme (GBM) is known to be one of the most malignant forms of brain cancer due to its resistance to chemotherapy. Hence, the identification of drugs able to impair GBM malignancy and aggressiveness is considered of great clinical relevance. Previously, we demonstrated that the specific activation of M2 muscarinic receptor, by the orthosteric agonist Arecaidine Propargyl Ester (APE), inhibits cell proliferation in a time and dose-dependent manner and it induces a severe apoptosis in human glioblastoma cell lines. Recently we have also analyzed the activation of M2 muscarinic receptor by N-8-Iper, a new dualsteric agonist with more potent binding efficacy than the orthosteric agonist APE. COP9 signalosome subunit 5 (CSN5) has been involved in the progression of diverse human cancers, including GBM. In the last years, CSN5 has received attention as a regulator of the degradation of cancer-related proteins through its de-NEDDylating activity toward cullin-RING ubiquitin ligases that regulates ubiquitin-proteasome system, suggesting its relevance in cancerogenesis. However, the biological functions and molecular mechanisms of CSN in glioblastoma remain poorly investigated. In this work we firstly characterized the role of CSN in GBM and the possible alteration of the ubiquitin-proteasome system after the M2 muscarinic receptor activation, by both M2 orthosteric and dualsteric agonists. Our data demonstrated that the M2 receptor activation, by both agonists, leads to a significantly decrease in NEDDylation, which is a post-translational modification with a key role in the ubiquitin-ligase activation. Alteration of this system may lead to an increasing oxidative stress and consequent cell death. In fact, our results demonstrate an increasing oxidative stress accompanied by increased activity of SOD and catalase enzymes activity after M2 agonist treatments. Moreover an alteration of the lipids and carbonylated proteins levels were also observed. Interestingly significant differences in mitochondrial morphology and respiration were also observed upon two M2 agonists treatment. These results suggest that the selective stimulation of these cholinergic receptors may significantly impair GBM cell activities compromising tumor cell survival.

T19-008D

Single-cell RNA and protein sequencing reveals functional heterogeneity of glioma-associated brain macrophages

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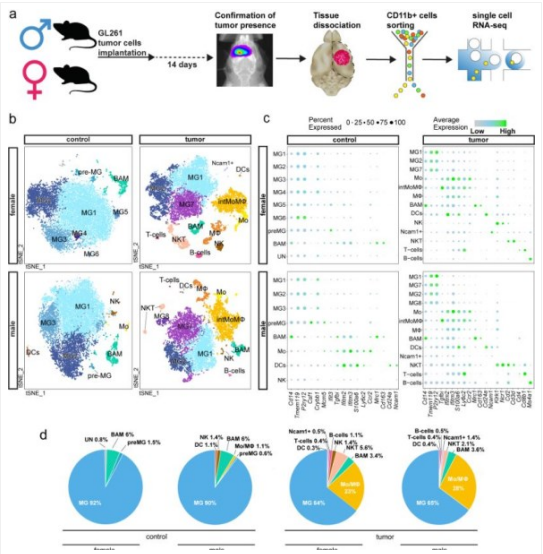
Microglia and bone marrow-derived macrophages accumulate and adapt a tumor-supporting role in human malignant gliomas that show prevalence in men. Brain resident microglia and peripheral macrophages have different origins and transcriptomic profiles that suggest distinct functions in tumorigenesis. However, the heterogeneity of functional cells/phenotypes and their specific roles in gliomas remain elusive. Here, we show scRNA-seq combined with surface protein labeling of CD11b+ cells sorted from experimental GL261 gliomas. The identified marker proteins showed distinct spatial distribution of identified subsets in glioma-bearing brains. We demonstrate that although microglia and monocytes/macrophages activate similar transcriptional networks within the tumor environment, the responses of monocytes/macrophages are stronger, and they are localized predominantly within the tumor core. Monocytes/macrophages show substantial transcriptional heterogeneity allowing to distinguish subsequent differentiation states. The differentiated macrophages upregulated immune-suppressive genes and exhibited a high level of PD-L1 protein, which points to their role in suppression of the immune response against the tumor. Additionally, we studied whether there are sex differences in the transcriptomes of myeloid cells infiltrating gliomas and found higher expression of MHCII encoding genes in glioma-activated male microglia. This finding was corroborated in bulk and scRNA-seq data from human diffuse gliomas. Our results suggest that sex-specific gene expression in glioma-activated microglia may be relevant to differences in incidence and outcomes of glioma patients.



Identification of immune cell populations in control and tumor-bearing brain hemispheres **a.** Scheme of the experimental workflow. **b.** t-SNE plot demonstrating clustering obtained for each group. Clusters annotations: MG – microglia, pre-MG – premature microglia, Mo – Monocytes, intMoMΦ – intermediate Monocyte-Macrophage, MΦ – macrophages, BAM – CNS border-associated macrophages, DCs – dendritic cells, Ncam1+- Ncam1 positive cells, NK – natural killer cells, NKT – natural killer T cells, B cells – B lymphocytes, T cells – T lymphocytes. **c.** Expression of "signature" genes. **d.** Pie charts demonstrating distribution of the identified cell types across samples.

T19-009D

Glioblastoma invasiveness and collagen secretion is enhanced by the endoplasmic reticulum compartmentalization of vitamin C

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Glioblastoma (GB) is one of the most aggressive tumors, with an average survival of 15 months after diagnosis and treatment. These tumors modify their metabolism, increasing the expression of glucose transporters, GLUTs, which incorporate glucose and other substrates, such as the oxidized form of vitamin C, dehydroascorbic acid (DHA). There is ample evidence that tumor cells preferentially uptake DHA, which can be generated through local

oxidation of ascorbic acid (AA), a process known as the bystander effect. Intracellularly, DHA is reduced to AA, a molecule that can be compartmentalized at the subcellular level. Interestingly, SVCT2, the AA transporter, appears to be expressed in tumors with a preferred intracellular localization. We hypothesized that GB cells preferentially take up DHA, which is intracellularly reduced and compartmentalized, promoting collagen biosynthesis and an aggressive phenotype. Our results show that glioblastoma cells express GLUT1, GLUT3, and SVCT2; they also take up DHA using GLUT1. GLUT3 and SVCT2 are preferably intracellular. Using a baculovirus system and reticulum enriched extracts, we detected that SVCT2 is mainly located in the rough endoplasmic reticulum and corresponds to a short isoform. AA was compartmentalized, stimulating collagen IV biosynthesis and its secretion. Vitamin C increased *in vitro* and *in situ* cell migration. Finally, tumors induced in immunocompetent guinea pig brains with U87MG (mesenchymal tumors) and HSVT-C3 (patient-derived tumors) cells showed that intracellular vitamin C deficiency retained collagen, reduced blood vessel invasion, and affected glomeruloid vasculature formation, all pathological conditions associated with malignancy. Based on our results, we propose a functional role of vitamin C in GB development and progression.

Acknowledgement

This work was funded by grants FONDECYT 1181243 (to Francisco Nualart), CONICYT-PIA ECM-12 (to Francisco Nualart), Fondecyt 1190848 (to Katterine Salazar), Fondecyt 11200335 (to Luciano Ferrada), and Fondecyt 11170959 (to Nery Jara). The authors thank Ximena Koch from the University of Concepción, Chile, and Birgit Jachnik, from the Department of Neurology, University Hospital Regensburg, Germany for their technical support. The authors thank Dr. Marjet Heitzer for the suggestion on the manuscript.

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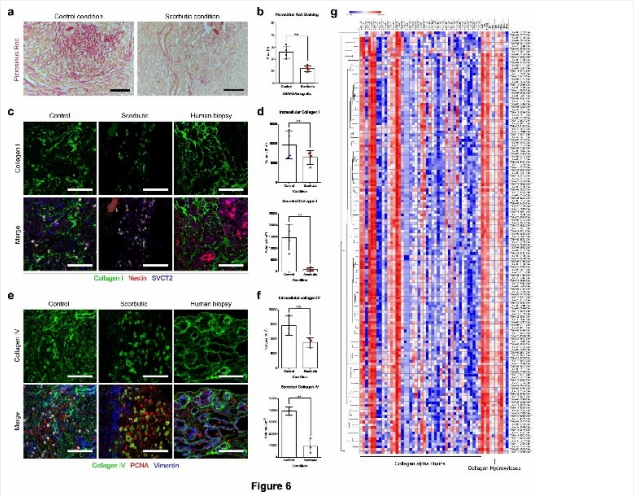

Figure 6

Vitamin C deficiency impairs extracellular collagen accumulation in glioblastoma.
a Picrosirius red staining (PRS) of U87MG xenografts (control and scorbutic Guinea pigs). **b** Quantification of the PRS (n = 3). **c** Immunofluorescence analysis (IFS) for collagen I (green) or collagen I / Nestin (NES) / SVCT2. **d** Quantitative analysis of intracellular and secreted collagen I. **e** IFS for collagen IV (green) or collagen IV / PCNA / vimentin (VIM). **f** Quantitative analysis of intracellular and secreted collagen IV. **g** Heatmap and hierarchical clustering of collagens and collagen hydroxylases (columns) gene transcription levels in 153 GB patient samples (rows) from TCGA database.

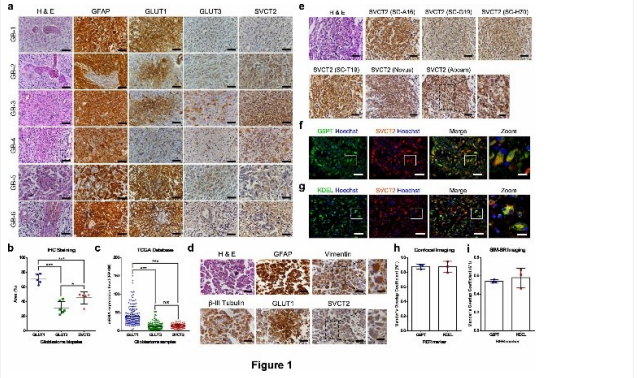

Figure 1

Human glioblastoma express SVCT2 intracellularly, which co-localizes with RER resident proteins.
a Hematoxylin (H&E) and immunohistochemistry staining (IHS)(brown) of glioblastoma (GB) patient biopsies. **b** Quantification of positive immunostaining. **c** TCGA database in 153 GB samples. **d** H&E and IHS (brown) of gemistocytes in the GB-5 biopsy. **e** H&E analysis and IHS (brown) of GB-2 to evaluate anti-SVCT2 reaction of different types of antibodies. **f, g** Immunofluorescence staining of GB biopsies showing SVCT2 intracellular colocalization with both ER markers. **h, i** Quantification of Mander's OC of SVCT2 over G6PT or SVCT2 over KDEL by both spectral confocal microscopy (**h**) and SIM-SR analysis (**i**).

T19-010D

Potential role of AQP4ex in edema associated high grade gliomas

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Glioblastoma Multiforme (GBM) is the most aggressive form of primary brain tumor with a tendency to invade surrounding healthy brain tissues. Accumulated evidence has suggested AQP4 as a key protein in glioma development. Indeed, findings of the role of AQP4 in cell migration suggest that AQP4 could be an important factor regarding glioma malignancies (1). In addition, AQP4 play an important role in the tumor associated edema coupled with blood-brain barrier (BBB) damage (2). Moreover, recent data suggest that AQP4 aggregation-state play a critical for the glioma cells fate (3). The aim of this study was to investigate the role of the new AQP4 isoform, AQP4ex, generated by translational readtrough mechanism, in GBM and in the tumor associated edema. To this purpose, 35 biopsies from GBM patients were analyzed to determine AQP4 expression levels, supramolecular aggregation and localization. Correlation with edema volume was obtained by MRI using the edema index (EI) parameter. Biopsies were firstly histologically characterized by hematoxylin and eosin staining in order to evaluate the presence and the extent of tumor tissue. Immunoblot experiments after SDS-PAGE and BN-PAGE were performed to evaluate the protein content and the supramolecular organization of AQP4 isoforms and immunofluorescence experiments were performed to assess the localization of AQP4ex and AQP4 in each GBM biopsy. Finally, BBB damage was evaluated by fluorescein levels in surgical specimens of GMB patients treated with the fluorescent dye used to guide-resection of gliomas. Data showed a strong reduction of the AQP4ex in tumor compared to the peritumoral regions. Interestingly, the reduction of the amount of AQP4ex caused an alteration of the supramolecular organization of AQP4 concomitant to a delocalization of AQP4 observed in the tumor region. Indeed, AQP4 lost its perivascular localization, and appeared mis-localized in tumor tissue and to some extent also in peritumoral region. Edema index correlated positively with the AQP4ex alteration levels suggesting an important role of AQP4ex in the accumulation of edema in the peritumoral area. Finally, fluorescein analysis revealed a moderate increase in fluorescein levels in the peritumoral region which conspicuously increased in the tumor region indicating a progressive damage of the BBB occurring during tumor development. Our study suggests a crucial role of AQP4ex in the glial alteration occurring in GBM correlated with tumor infiltration and vasogenic edema.

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T19-011E

The effects of H3.3 K27M-mediated epigenetic dysregulation on murine glial development *in vivo.*

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Diffuse intrinsic pontine glioma (DIPG) is an incurable high-grade glioma arising in the brainstem primarily of younger children. DIPG prognosis remains poor due to the unresectable tumor location and its resistance to radiation and available therapeutics, emphasizing the need for better understanding of the underlying developmental context and pathogenesis of DIPG to identify new therapeutic vulnerabilities. Lysine 27 to methionine mutations (K27M) occur in histone H3 at a high frequency (~80%) of DIPGs and other pediatric diffuse midline gliomas (DMG). The high frequency of K27M mutations and their exclusivity to DIPG and DMG suggests K27M has unique selective advantage in a distinct spatiotemporal setting for tumorigenesis. K27M causes a dominant loss of the repressive H3K27me3 resulting in epigenetic dysregulation involving release of bivalent promoters. Our group developed a genetically engineered mouse model harboring a conditional knock-in of K27M into the endogenous *H3f3a* locus which offers the opportunity to study K27M mutations in a physiologically and developmentally relevant manner. With this model, we showed K27M cooperates with Pdgfra activation and/or p53 loss to drive spontaneous gliomagenesis selectively to brainstem and hindbrain locations illustrating its regional selectivity. Single-cell RNA-seq studies of human K27M DIPGs reveals oligodendrocyte progenitor-like cells as the main proliferative and stem-like population present in DIPGs. *In vitro* assays from our group and others indicate K27M alters stemness and development of neural stem cells and glia. We have also shown that depletion of K27M in patient-derived xenografts induces oligodendrocyte differentiation signatures, reduces stemness and proliferation signatures and decreases tumor growth. In total, previous studies indicate a role for K27M in regional selectivity of brainstem tumorigenesis along with altered glial differentiation, promoting a progenitor-like state. However, it remains unclear why K27M-mediated epigenetic dysregulation exhibits regional selectivity and how K27M alters glial development *in vivo*. Here, we assess region-specific effects of H3 K27M alone throughout early postnatal glial development *in vivo* via immunofluorescent staining and qPCR of neural stem/progenitor, glial progenitor, mature glial, and proliferation/cell death markers in our K27M knock-in mouse model. The results from these studies will provide important insights into the impact of epigenetic dysregulation on glial development, and the cell-state dependent effects and regional selectivity of K27M-mediated epigenetic dysregulation to inform developmental and cellular origins of DIPG.

T19-012E

Targeting the interleukin 10 receptor complex using a transmembrane targeting peptide to control microglia / macrophage polarisation in glioblastoma.

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Microglia and macrophages are innate immune cells with critical roles in several pathologies. However, they can exert different and opposite effects, insofar as they can adopt different polarisation states. These polarisations present two theoretical extremes called M1 and M2. M1 macrophages, also called activated macrophages, have a pro-inflammatory phenotype whereas M2 macrophages, called alternatively activated macrophages, have an anti-inflammatory phenotype. Although the cells do not endorse one or the other polarisation, they are situated on a continuum between both. In glioblastoma, among the several cells present in tumour microenvironment, tumour-associated macrophages and microglia play an important role in tumour growth and maintenance. Their activation state is akin the M2 anti-inflammatory phenotype. In consequence, an immunosuppressive mechanism is established and the anti-tumoral immunity is repressed.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine exerting its role via IL-10 receptor (IL-10R), a complex constituted of multimers of two α subunits and two β subunits. Its activation leads to the transcription of anti-inflammatory genes via STAT3 phosphorylation and translocation. IL-10 is implicated in the differential polarisation of microglia and macrophages.

We have developed, using prediction tools, a peptide mimicking and targeting the transmembrane (TM) domain of the IL-10R subunit β. This membrane targeting peptide (MTP-IL10Rβ) is able to restrain IL-10R multimerization at its TM domain by creating a steric hindrance. Thus, it is limiting the activation of the receptor and its anti-inflammatory effect. Hence, the activation of macrophages and microglia is counterbalanced so they can exert their pro-inflammatory, anti-tumoral response. We showed *in vitro* that MTP-IL10Rβ limits the activation of the receptor and its downstream signalling pathways using proximity ligation assay, western-blot, ELISA and RT-qPCR. We also showed that it is capable of modulating polarisation. This project constitutes a new opportunity for the modulation of macrophage polarization with therapeutic potential.

T19-013E

Characterization of CD271^{+} Schwann Cell as *in vitro* model of schwannomatosis.

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Schwannomas are the most common type of peripheral nerve tumor, originating from a clonal population of Schwann cell (SCs). In most cases, schwannomas are sporadic, but some are associated with neurofibromatosis type 2 (NF2) or schwannomatosis (SWN). One of the cause of the transformation of SCs into schwannomas is the inactivation of the neurofibromin 2 (*NF2*) gene and the consequent loss of function of the tumor suppressor protein Merlin. Two other genes *SMARCB1* and *LZTR*1 have been identified as possible predisposition genes for schwannoma development. However, from a pathogenomic point of view, it is not excluded that mutations in other genes, not yet identified, may occur. The identification of these genes and/or cellular mechanisms at the basis of SWN development was limited by the lack of *in vitro* cell models. In this study, thus, primary human SCs, derived

from schwannomas, were isolated by a positive immunomagnetic cell isolation system, based on their surface expression of NGF receptor p75 (CD271). These cells were characterized by flow cytometry analysis of different tumor cell suspensions: tumor digestion, pre- and post-purification specimens. Before purification, the $CD271^+$ cells were less than 10%, confirming the cellular heterogeneity of the tumor. Analysis for CD45, a marker of immune-derived cells, showed about 40% of these cells in the pre-purification culture. Then, purification led to an enrichment of 80% in $CD271^+$ SCs, as confirmed also by the IFL analysis of the SCs marker S100.

Overall, the human SCs cultures herein described represent an excellent *in vitro* model for wide genome screening and genomic editing studies. The characterization of cells from patients with different clinical presentation, by means of genotype-phenotype correlation studies, will make possible the identification of new molecular pathways and/or genes potentially linked to SWN.

Acknowledgement

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T19-014E

Feasibility of photodynamic therapy of glioblastoma multiforme with Tetramethylrhodamine methyl ester.

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Glioblastoma Multiforme (GBM) is the most aggressive brain cancer with abysmal prognosis. According to the WHO classification, GBM is a grade IV infiltrating glioma. The introduction of the “Stupp protocol” in 2005 (chemotherapy with one specific drug, temozolomide, combined with radiotherapy) has been the only major milestone towards improving the outcome for GBM patients but, at best, it prolongs survival by ~6 months. After removing the bulk of the superficial tumour it almost always re-occurs from areas immediately adjacent to the primary lesion because of infiltration of the apparently healthy tissue by residual GBM cells. Suppression of this infiltration would greatly improve the chances of longer-term survival. Here photodynamic therapy (PDT) might prove useful because it is less traumatic than direct surgical ablation of the tissue. PDT is defined as a technology where a specific photosensitive chemical is loaded into cells and, upon illumination with light of appropriate wavelength, causes photochemical reactions which eventually kill the targeted cells. Previous trials of PDT for GBM all used one specific photosensitiser (PS), 5ALA, but did not produce convincing effects. This is not surprising because 5ALA is a very poor PS, even though it rather specifically accumulates in GBM cells.

We found that a chemical previously used for imaging mitochondrial membrane potential, tetramethylrhodamine methyl ester (TMRM), can be used as a highly effective PS. TMRM in low to medium nanomolar concentrations (<300 nM) rapidly accumulates in mitochondria of GBM cell lines and, when illuminated by green light (530-550 nm), triggers immediate depolarisation of the mitochondria. Mitochondrial depolarisation is a recognised signal for the opening of the mitochondrial transition pore and subsequent release of apoptotic signals. PDT with TMRM was tested on 6 patient-derived GBM cell lines. In all cases TMRM-PDT led to suppression of cell proliferation and strong loss of viable cells within 3 days. 5 of 6 GBM cell lines had much higher basal mitochondrial potential compared to normal rat astrocytes used as a reference. This resulted in greater load with TMRM and, presumably,

greater photodynamic effect. In addition, healthy astrocytes were able to recover their mitochondrial potential after 24 hours, while GBM cells were not. As such TMRM is not toxic to cultured cells at concentrations at least 10 times greater than used for PDT. In addition, we found that, at least in two GBM cell lines (UP007, GIN27), activation of adenylyl cyclase with NKH 477 can enhance the impact of PDT. After a 24 hour pre-incubation with 10μM NKH the effect of PDT was potentiated.

We propose to further explore the usability of TMRM for PDT of GBM and possibly other cancers.

Acknowledgement

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T19-015E

A Cx43 peptide inhibits metabolic plasticity in human glioma stem cells while sparing human neural stem cells and astrocytes

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Glioblastoma is the most aggressive primary brain tumour and has a median survival of 16 months. Inhibition of c-Src activity in glioblastoma stem cells (GSCs, responsible for glioblastoma lethality) and primary glioblastoma cells by the cell-penetrating peptide TAT-Cx43$_{266-283}$, based on Connexin43, reduces tumorigenicity and boosts survival in preclinical models (ref. 1). Because c-Src can modulate cell metabolism and several reports revealed poor clinical efficacy of various antitumoral drugs due to metabolic rewiring in cancer cells, here we explored the inhibition of advantageous GSC metabolic plasticity by the c-Src inhibitor TAT-Cx43$_{266-283}$. The Connexin43 peptide TAT-Cx43$_{266-283}$ decreased glucose uptake in human GSCs and reduced oxidative phosphorylation without a compensatory increase in glycolysis, with no effect on brain cell metabolism, including rat neurons, human and rat astrocytes, and human neural stem cells. TAT-Cx43$_{266-283}$ impaired metabolic plasticity, reducing GSC growth and survival under different nutrient environments. Finally, GSCs intracranially implanted with TAT-Cx43$_{266-283}$ showed decreased levels of important metabolic targets for cancer therapy, such as hexokinase-2 and GLUT-3. This is especially relevant since both HK-2 and GLUT-3 are necessary for the development of glioblastoma in pre-clinical models and are associated with decreased overall survival in glioblastoma patients. The reduced ability of TAT-Cx43$_{266-283}$ treated GSCs to survive in metabolically challenging settings, such as those with restricted nutrient availability or the ever-changing in vivo environment, allows us to conclude (ref. 2) that the advantageous metabolic plasticity of GSCs can be therapeutically exploited through the specific and cell-selective inhibition of c-Src by TAT-Cx43$_{266-283}$.

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T20 | Glial diversity

T20-001A

In vitro heterogeneity of human oligodendroglia

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Introduction: Oligodendroglia in the CNS are a heterogeneous population demonstrating morphological differences[1], distinct features in the brain and spinal cord[2], and a dual role in facilitating fast conduction of action potentials while providing metabolic support to the underlying neuronal axons[3]. We have recently shown that adult brain human oligodendroglia are heterogeneous at the transcriptome level, with some oligodendrocytes expressing more myelin gene transcripts than others, suggesting better myelinating capacity, while the proportions of these are altered in Multiple Sclerosis[4].

In order to be able to model different oligodendrocytes functions, we decided to investigate whether human embryonic stem cell (hES) derived oligodendroglia in culture display a similar heterogeneity, using monolayer oligodendroglia cultures, 3D brain organoid cultures, and transplantation of 2D oligodendroglia into Shi/Shi: $Rag2^{-/-}$ mice Figure 1).

Results: Transcriptional analysis of single-cell RNA sequencing data shows that hES-derived oligodendroglia grown in a monolayer are a heterogeneous population that can be divided into distinct oligodendrocyte clusters. We also detected the presence of neuron, astrocyte and pericyte clusters. We validated this heterogeneity using in situ hybridization and immunofluorescence for unique or enriched RNA markers of individual clusters within the oligodendrocyte lineage, as identified in a post-mortem human brain dataset. We performed canonical correlation analysis (CCA) to map our hES-derived oligodendroglia transcriptional data onto our post-mortem adult oligodendroglia single-nuclei RNA sequencing data, as well as with other datasets. This showed the presence of similar distinct oligodendrocyte 'states', albeit with high numbers of $SOX2^{+}$ immature cells. This heterogeneity is maintained in cortical organoid cultures and also after transplantation of hES-derived OL into Shi/Shi:$Rag2^{-/-}$ mouse brains. Heterogeneity between these samples has also been compared. These findings will aid in the discovery of the different functional roles of human oligodendrocytes.

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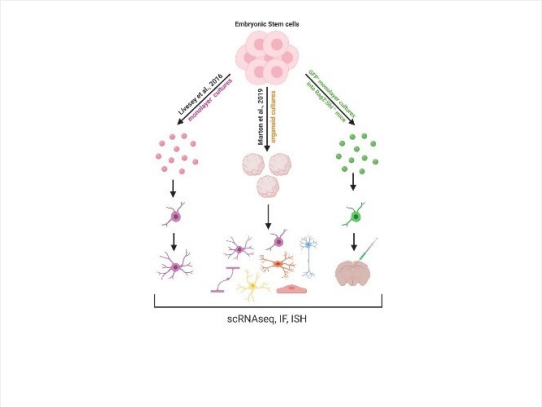

hES-derived OL models employed

T20-002A

Functional identity of a new subtype of astrocyte in the mouse: the Olig2-astrocytes

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In the vertebrate central nervous system, macroglial cells, oligodendrocytes and astrocytes, display highly distinctive morphological and functional properties. Astrocytes participate in a wide variety of complex and essential functions, including synaptic development and plasticity,trophic regulation and blood-brain barrier formation. For the last twenty years, the attention given to those cells has grown, permitting to unravel aspects of their development and in particular to reveal the existence of an unexpected heterogeneity whose functional significance remains to be defined. Our recent work led us to evidence a previously unidentified sub-population of astrocytes, named Olig2-AS, in the mouse spinal cord that can be distinguished from other astrocyte populations by the expression of Olig2, a transcription factor currently recognized as a hallmark of oligodendrocytes (Ohayon et al., 2019). To address the question of the functional specificity of this astrocyte subtype, we performed geneprofiling of the Olig2-AS. Those studies are indeed crucial to provide insights into astrocyte diversity either at the molecular or functional level. By using a double reporter mouse strategy(Aldh1L1-GFP x Olig2-tdtomato), we have been able to color code specifically Olig2-AS in the spinal cord, leading to an efficient FACS-sorting of the glial cells and RNA-seq based segregation analysis. The ongoing analysis of the Olig2-AS transcriptome has permitted us to identify new markers for the Olig2-AS population and provide insights into astrocyte diversity at the functional level. Gene ontology analysis suggests that Olig2-AS might be astrocytes specialized in regulation of the synaptic activity compared to the other astrocytes. Together, our data indicate that in the spinal cord, the Olig2-AS are a molecularly and functionally distinct subpopulation of astrocytes.

T20-003A

Lifelong structural dynamics of cortical oligodendrocytes

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Oligodendrocytes play an important role in neural circuit function by forming myelin, which supports rapid and energy-efficient conduction of neural impulses. Over the lifespan, oligodendrocytes are continuously supplied by de novo synthesis from the precursor cells, and at least in part, remodel their existing subcellular cytoarchitecture. In this work, we aim to understand the lifelong structural dynamics of cortical oligodendrocytes. To fully capture the structure of individual oligodendrocytes, we introduced a tissue clearing/expansion technique, which offered unambiguous identification of subcellular details in 3-dimension. We acquired comprehensive volumetric imaging over the mouse somatosensory cortex, and tracked the full skeletons of >150 oligodendrocytes (>8000 processes, >17000 myelin) across the postnatal ages of 20–640 days. To efficiently handle this large dataset, we exploited the graph theory and represented each oligodendrocyte as a graph, a set of vertices connected by edges. Harnessing a deep learning algorithm, we transformed each graph into a summary vector with a fixed size and performed unsupervised clustering. Consequently, we obtained a set of morphological subgroups of cortical oligodendrocytes. By further incorporating the temporal information (postnatal age), we revealed the age-dependent population shift among the morphological subgroups.

Acknowledgement

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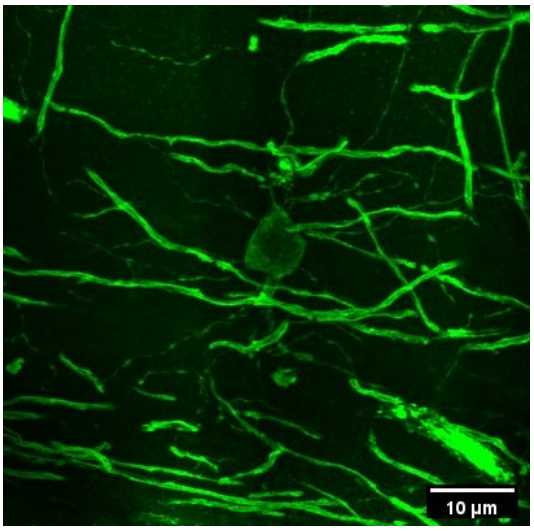
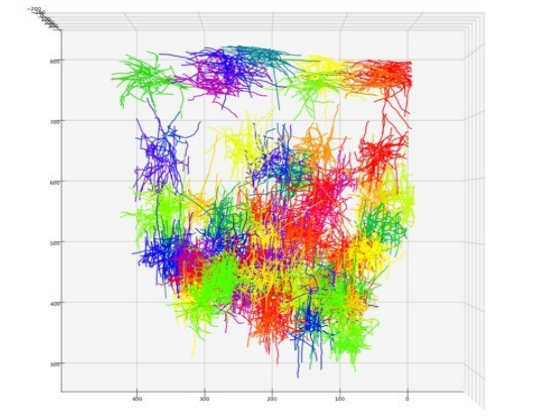


oligodendrocyte in expanded tissue
Representative image of 2D projected oligodendrocytes in expanded tissue



3D reconstruction of oligodendrocytes in somatosensory cortex
3D morphology of oligodendrocytes using tissue expansion technique from somatosensory cortex. Almost cells in the volume (450 x 450 x 130 um^3) were reconstructed.

T20-004A

Ultrastructural assessment of NG2-expressing cells in the perisynaptic region of the ventral horns in spinal cord injury

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Recently, glia expressed neuron-glial antigen 2 (NG2) proteoglycan has received special attention in studies of the mechanisms of plasticity and regeneration in the central nervous system. NG2 glial cells are the smallest (according to data from 3 to 10%), but as it turned out, no less significant population of cells. In this work, the expression and distribution of NG2 proteoglycan were investigated by immunoelectron microscopy (Hitachi HT7700, Tokyo, Japan) in the ventral horns (VH) of an intact and injured rat spinal cord, and the colocalization of NG2 and astrocyte marker aldehyde dehydrogenase 1 L1 (ALDH1L1) was shown.

In the intact spinal cord NG2 was detected in myelin membranes and the cytoplasm of oligodendrocytes. NG2 glia with thick and short processes, irregular shaped cell bodies with polymorphic nuclei and explicit expression NG2 proteoglycan were observed in the perisynaptic region. In abovementioned cells, expression of ALDH1L1 was absent. A significantly lower NG2 expression was detected in the cytoplasm and membranes of ALDH1L1$^+$ astrocyte processes surrounding the neuron or located in the perisynaptic region. In the above mentioned areas, at all studied distances in the VH, by 7 days after SCI, astrocyte immunoreactivity to ALDH1L1 increases, which, along with an increase in GFAP expression, is a sign of their pronounced reactivity. At different distances (3-5, 6-8 and 10-12 mm) from the epicenter of injury in the caudal direction at 7 and 30 days post injury, astrocyte immunoreactivity to ALDH1L1 increased, which, along with an increase in GFAP expression, is a sign of their pronounced reactivity. The cytoplasm of reactive astrocytes was electron-clear and not contained fibrous structures characteristic of the intact astrocytes processes. The expression of NG2 appears to be increased both in NG2 glia and reactive astrocytes at 7 and 30 days after spinal cord injury (SCI) in the VH located caudally from the epicenter of the injury.The results indicate the presence of NG2-expressing astrocytes in the intact spinal cord, and the ability of reactive astrocytes after SCI to produce NG2, the shedding ectodomain of which affects restoration of neural networks.

Acknowledgement

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T20-005A

Ependyma: a new target for AQP4-IgG in NMO?

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Background: Ependyma maintains crucial functions such as the regulation of cerebrospinal fluid (CSF) circulation by synchronous ciliary beating, the monitoring of molecular exchanges between CSF and parenchyma and the

regulation of neural stem cells (NSCs) proliferation and survival present in the subventricular zone (SVZ).
Neuromyelitis Optica (NMO) is a severe neurological disease associated with autoantibodies (NMO-IgG), directed against aquaporin 4 (AQP4). NMO-IgG are present in patient's CSF during attacks, and are known to trigger astrocyte dysfunction leading to demyelination and axonal loss. Interestingly, ependymal cells also express AQP4 and evidences of ependymal alteration are reported in NMO.

Objectives: To evaluate if NMO-IgG target ependymal cells, and lead to morphological changes and dysfunctions of ependyma.
Methods: NMO-IgG were purified from AQP4 antibodies positive patients' plasma. IgG from healthy donors (CTRL-IgG) and non-treated conditions were used as controls. To study the effects of NMO-IgG, two models of ependyma were used: primary ependymal cell cultures and cultured wholemount dissections ("en-face" view of the entire ependyma) from adult rat lateral ventricular. After 24h treatment with NMO-IgG or control conditions, immunolabeling of specific proteins (AQP4, cilia and NSCs), and ependymal flow assay with fluorescent microbeads were used to evaluate morphological and functional changes.
Results: We showed that NMO-IgG: 1) induced agglomeration of AQP4 and the gap junction connexin-43 at the lateral membrane of ependymocyte, and increased cell size; 2) induced cilia shape alterations (**Figure1**), planar apical depolarization of cilia tufts and altered the speed and directionality of beads injected on the surface of wholemounts; 3) activated the subventricular NSCs by increasing the number of proliferating cells and the number of quiescent NSCs processes contacting the CSF.
Conclusions: These results show that NMO-IgG directly induce alterations of ependymal cells' functions and suggest an involvement of ependyma in NMO physiopathology.

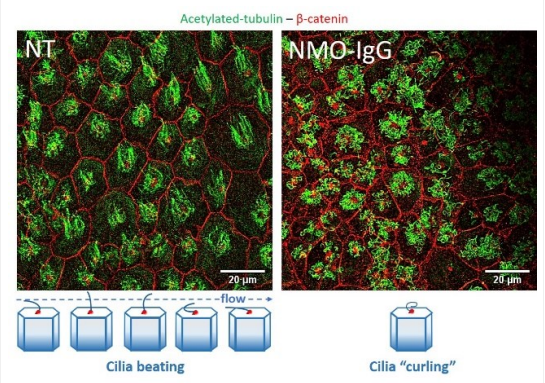

Figure1: NMO-IgG alters ependymal cilia shape. Primary cultures of ependymal cells were treated during 24h with NMO-IgG and then fixed for immunolabeling of cilia with acetylated-tubulin (green) and lateral membranes with β-catenin (red). NT: non-treated.

T20-006B

Yin Yang 1 sets up the stage for cerebellar astrocyte maturation

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Diverse subpopulations of astrocytes tile different brain regions to accommodate local requirements of neurons and associated neuronal circuits. These cells metabolically supporting neurons, maintaining ion balance, regulating concentrations of neurotransmitters, regulating the blood brain barrier, reinforcing and pruning synapses, guiding migrating neurons, and aiding with immune function. Nevertheless, molecular mechanisms governing astrocyte diversity remain mostly unknown. We explored the role of a zinc finger transcription factor, Yin Yang 1 (YY1), that both activates and represses transcription and regulates cell-type-specific loops of active chromatin. YY1 is expressed in astrocytes. Specific deletion of YY1 from astrocytes (*Yy1*$^{\Delta AST}$) causes severe motor deficits in mice, induces Bergmann gliosis, and results in loss of GFAP expression in velate and fibrous cerebellar astrocytes. We found that YY1 is dispensable for the initial stages of astrocyte development with normal morphology and distribution of GFAP-positive cells, including Bergmann glia, velate astrocytes, and fibrous astrocytes in *Yy1*$^{\Delta AST}$ mice at postnatal day 10 (P10). Interestingly, YY1 controls subpopulation-specific gene expression during astrocyte maturation with surprisingly diverse effects in distinct layers of the cerebellum. Single cell RNA-seq analysis indicated that YY1 exerts specific effects on gene expression in subpopulations of astrocytes. Additionally, YY1 is continuously required to maintain astrocyte identity even in the adult cerebellum. Our findings suggest that YY1 plays critical roles regulating cerebellar astrocyte maturation during development and maintaining a mature phenotype of astrocytes in the adult cerebellum.

T20-007B

Monitoring astrocyte diversity by a cell-type specific proteomic approach

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Astrocytes and neurons form a tight partnership in the brain with astrocytes not only supporting neuronal function in manifold ways but also modulating neuronal activity. Compared to neurons, the structural and functional diversity of such astrocytes were only poorly understood. To understand their diversity *in vivo*, the application of Aldh1l1 as a pan-astroglial marker has resolved the methodical hurdles and paved the way to cell-type-specific studies. We developed an astrocyte-specific metabolic labelling strategy using Tamoxifen-inducible Aldh1l1-*Cre*/ERT2 mice, enabling the highly specific expression of the L274GMetRS in this cell-type. The subsequent application of the Non-Canonical Amino Acid Tagging (NCAT) technology *invivo* enabled us to study newly synthesized proteomes as a readout of their translational diversity within four different regions of the mouse brain.

The proteomic data reveals a robust set of proteins specifically expressed in astrocytes when compared with multiple datasets of both proteomic and transcriptomic origin. Subsequent gene enrichment analysis based on Gene Ontology and KEGG shows that the proteome of astrocytes is essentially involved in metabolic processes and its main part is statistically unvarying between the brain regions under homeostatic steady-state conditions. Nevertheless, we identified in each of these proteomes a set of proteins that are highly specific for the respective astrocytes analysed in the different brain regions.

A more detailed investigation into translational rates of astrocytes in sub-regions of the hippocampus added supporting results. The astrocytes in the CA1 region exhibit significantly higher translational rates than CA3 and DG regions. Correspondingly, astrocytes in the CA1 also showed significant morphological diversity compared to the DG region. Besides, the layers within the CA1 region further yielded a different view as the *Stratum Oriens and Stratum Pyramidale* layer has a significantly high translation rate compared to the rest of the layers.

Thus, this work presents an *in vivo* approach for the cell-type-specific analysis of the proteomes from astrocytes situated in different regions of the brain and its diversity in subregions within the hippocampus.

Acknowledgement

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T20-008B

Regional astrocytic heterogeneity in models of Parkinson's disease

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Diversity of astrocytic structure and function is becoming increasingly recognised[1,2], but its roles in disease progression as well as translational perspectives arising from this knowledge are not well understood. To address this topic, we set out to investigate the regional differences between rat astrocytes cultured from 8 different brain regions: cortex, olfactory bulbs, hippocampus, striatum, thalamus / hypothalamus, midbrain, brain stem, and cerebellum.

Single-cell calcium analysis revealed that signalling patterns as well as activity of specific calcium channels varied based on the brain region. We also found that the gap junctional connectivity within astrocytic networks differed, as did the expression of gap junctional proteins. Finally, patterns of mitochondrial-glycolytic respiration ratios also

varied, with hindbrain regions relying on mitochondrial function to a greater extent.

Parkinson's disease (PD) is known to progress in a stereotypical regional pattern described by Braak stages[3], and the reasons for selective susceptibility of particular regions of the brain to cell death or Lewy body accumulation are still under investigation[4]. It is likely that neuronal diversity cannot explain the full extent of the pathology progression, so we hypothesised that astrocytes would also have different levels of susceptibility to alpha-synuclein lesions similar to those found in PD brains.

We found that astrocytes from different brain regions varied in their response to the alpha-synuclein pre-formed fibrils (PFFs). For instance, cortical astrocyte-containing cultures responded by releasing increased amounts of IP-10 cytokine, while midbrain-astrocyte containing cultures upregulated release of RANTES. Both midbrain and cortical astrocytes responded to the PFF challenge with decreased cytosolic calcium level after ATP stimulation, but oscillatory patterns differed.

To overcome the pathology induced by alpha-synuclein PFFs, we are testing a novel treatment, which acts specifically on astrocytic networks. It reduced the abnormal release of inflammatory cytokines and helped normalise calcium signalling that was affected by alpha-synuclein, especially in rat and human cortical astrocytes. If successful in further pre-clinical trials, this treatment will offer a promising new perspective in PD therapeutic approaches that is independent from dopamine replacement.

Acknowledgement

We would like to express gratitude to Dr Joana Tavares (University of Cambridge, Department of Clinical Neurosciences) for her advice on cellular respiration assays, and Dr Matthew Wayland (University of Cambridge, Department of Zoology) for his assistance with calcium imaging. We also want to thank our colleagues at the Cambridge Stem Cell Institute Imaging Core Facility, NIHR Cambridge BRC Cell Phenotyping Hub, and the Department of Pharmacology for the access to the imaging equipment necessary to conduct our experiments. We would like to acknowledge financial support of the project by Addenbrooke's Charitable Trust and Ferblanc Trust.

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T20-009B

Spatial distribution of oligodendrocyte populations within the mouse central nervous system

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We have previously identified transcriptionally distinct oligodendrocyte populations[1,2], the spatial distribution of which within the central nervous system is not yet to be completely defined. Using RNAScope and in situ sequencing (ISS) of juvenile and adult mouse brain and spinal cord, we have started to uncover age- and region-specific distributions of major oligodendrocyte populations[3]. The abundance of Mature oligodendrocytes type 2 (MOL2), type 5 and 6 (MOL5/6) increases with age in cortex and corpus callosum, while the abundance of Mature oligodendrocytes type 1 (MOL1) decreases in both analyzed brain regions. Here we perform additional ISS analysis of corpus callosum and cortical layers I-VI, which reveals differences in respective oligodendrocyte populations' contribution to these regions. In the spinal cord, MOL2 and Mature oligodendrocytes type 3 (MOL3) seem to preferentially reside within the white matter, contrary to MOL5/6, which are specific to grey matter. Oligodendrocyte lineage maturation and function might be specified by the local environment or interactions with different types of neurons, glia, and immune cells, in particular in the context of disease. To investigate the changes in the spatial allocation of oligodendrocyte populations related to their neighboring glial and immune cells in disease, we are currently applying ISS to the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis. Adding a spatial dimension to the single-cell RNA sequencing data might be a step towards a better understanding of oligodendrocyte heterogeneity.

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T20-010B

Abnormal microglia development and function in Down Syndrome revealed by human iPSC-based microglial chimeras

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Down syndrome (DS), the main genetic origin of intellectual disability, is caused by trisomy of human chromosome 21. A number of containing genes that are involved in related to the regulating immune defects functions are located on human chromosome 21. Microglia, the resident macrophages of the CNS, are essential for proper brain development through synapse pruning, neurogenesis, and differentiation. However, it is largely unknown whether and how trisomy chromosome 21 alters the development of human microglia in DS. By using our newly developed Generating human induced pluripotent stem cell (hiPSC)-based microglial chimeric mouse models by in which we transplanting transplanted primitive macrophage progenitors from DS and healthy control hiPSC into neonatal mouse brains, we found that DS microglia showed decreased complexity and length of processes as well as increased synaptic pruning function. Importantly, DS microglial chimeric mice exhibited alter synaptic neurotransmission in the hippocampus, as compared to control microglia chimeric mice. Further single-cell RNA-sequencing analysis of the DS and control microglia chimeric mouse brains will provide new insights into the mechanisms underlying the alter DS microglia development and abnormal functions. Results from our study using a powerful, new hiPSC microglial chimeric mouse model may lead to the identification of potential molecules that can be targeted to improve microglial function in DS, providing a new therapeutic avenue for the treatment of DS.

T20-011C

Roles of axon tract-associated embryonic microglia in forebrain development

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Brain functioning relies on complex neural circuits that begin to assemble during embryogenesis and alterations to these processes can lead to neurological or psychiatric disorders. Microglia, the brain resident macrophages, colonize the brain parenchyma during early embryogenesis and contribute to homeostasis in physiological conditions as well as in response to insults, through phagocytosis and secretion of a broad panel of factors. In addition to their immune functions, microglia have been identified over the past years as regulators of neural circuit formation and activity. Furthermore, recent single cell RNA-sequencing studies have revealed contrasting microglial transcriptional substates throughout development and postnatal life, associated with specific functional and pathophysiological processes. In particular, a distinct substate of axon tract associated microglia (ATM) has been shown to regulate postnatal oligodendrocyte populations and myelinogenesis. Remarkably, during embryogenesis, microglia are particularly associated with developing forebrain axon tracts and are known to regulate dopaminergic axons outgrowth and callosal axons fasciculation. In this study, we show that embryonic microglia associating with axons already display morphological, molecular and phagocytic profiles resembling postnatal ATM. Our ongoing work investigates the processes that regulate the emergence of this embryonic ATM substate as well as their roles in the developing forebrain.

T20-012C

Single-cell RNA Sequencing unveils an unprecedented molecular and functional heterogeneity of cerebellar astrocytes

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In the cerebellum, astrocytes are characterized by a peculiar heterogeneity, and are fundamental for the correct development and functioning of this brain area. However, the ontogenesis of such astroglial diversity remains poorly explored and little is known about the specific molecular identities and functions of each cerebellar astrocyte type. Recently, by combining *in vivo* clonal analyses with proliferation/birthdating studies, we demonstrated that a spatially and timely regulated developmental program drives cerebellar astrogliogenesis in mice. The exploration of

publicly available single-cell RNAseq data of adult mouse cerebella revealed novel molecular and functional specializations of distinct astrocyte subtypes. While the genes they express in common support their joint, broad, roles in synapse function and modulation, Gene Ontology and pathway analyses performed on differentially expressed genes showed divergent profiles of Bergmann glia and parenchymal astrocytes, likely reflecting specific interactions with defined cerebellar circuits. Furthermore, within each major astrocyte subtype, the cellular localization within the cerebellar territory in the vermis vs hemispheres or in distinct cerebellar lobuli emerged as a prominent driving force in their transcriptional heterogeneity. Ongoing analyses will clarify the functional significance of this peculiar spatial pattern, as well as the developmental trajectories of distinct astrocytes subtypes, with the goal of identifying the molecular determinants of cell fate switches and differentiation.

Acknowledgement

Our work was supported by Fondazione Umberto Veronesi and IBRO-PERC, for V. Cerrato's research stay in the laboratory of Prof. Ludovic Telley (Univ. of Lausanne, Switzerland).

T20-013C

Dissecting the links between cell shape, glial function and signaling dynamics *in vitro* using human induced pluripotent stem cell-derived astrocytes

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Astrocytes are glial cells with a dynamic and complex morphology, characterised by multiple primary processes that split into branchlets and then ramify into thousands of fine peripheral processes. These finer structures are the points of contact between both adjacent astrocytes and synapses, so their number, length and characteristics are key to overall astrocyte functions. Furthermore, astrocyte shape and process organisation is very diverse and changes depending on subtype, brain region and in response to changes in microenvironment activity. Additionally, astrocyte morphology alterations occur with the reactivity, alongside in neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS). So far, there has been a lack of systematic studies that investigate how the diversity of astrocyte shape is linked to their performance in key metabolic and signaling functions, or how differing morphologies due to reactivity or disease-associated mutations correlate to changes in function. To address this, we performed a systematic characterisation using high-content imaging, semi-automated image analysis, human induced pluripotent stem cell (iPSC) differentiation and genetically encoded indicators (GECIs) to investigate how astrocyte shape is linked to fundamental functions, such as intercellular signaling and metabolic dynamics. We have generated iPSC-derived astrocytes with differing morphologies using directed differentiation with developmentally relevant cytokines, bone morphogenic protein 4 (BMP4) and ciliary neurotrophic factor (CNTF), commonly used to produce astrocytes *in vitro*. To obtain quantitative information on astrocyte shape at the single-cell level, we developed a semi-automated image analysis pipeline using machine learning and morphometric analysis software to batch analyse thousands of cells. Combined with multivariate analysis, we determined the timepoint of shape divergence between BMP4 and CNTF treated astrocytes and identified subpopulations based on shape descriptors, alongside mitochondrial and cytoskeletal dynamics with fluorescent GECIs. Using this pipeline we also demonstrated the impact of neuronal interactions, reactivity and ALS-associated mutations on changing BMP4 and CNTF-astrocyte shape. Additionally, we used soft lithography with varying topographies, such as microgrooves and microwells, to examine how physical features can alter shape and molecular mechanisms.

Overall, we applied methods of generating functional astrocytes with differing morphologies to examine how astrocyte biophysical properties, such as shape, influence vital metabolic and signaling dynamics. These findings provide insight and a systematic characterisation into the significance of astrocyte shape changes in health, reactivity and disease.

Acknowledgement
Thank you to our collaborators in the Patani group and The Making Lab at The Francis Crick Insitute, and the Medical Research Council for funding this project.

T20-014C

How cellular morphology shapes functional organization of calcium signaling in astrocytes

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Astrocytes' complex branched anatomy is increasingly regarded as a structural component of the cell's functional specification. On the other hand, there is no understanding of whether and to what extent the astrocyte's anatomy affects the cell's functional readout in terms of its intracellular calcium signaling. We thus set out to deploy quantitative characterization of branching anatomy of hippocampal astrocytes, using descriptive statistics for multidimensional morphology data. Unsupervised clustering is then deployed to identify putative structural signatures for different categories of astrocytic anatomy. Based on such categories, we deploy generative modeling to grow different lines of astrocytes in silico, each characterized by its statistics of morphological parameters. Different experimental-like protocols then probe the artificial astrocytes to seek a characterization of the spatiotemporal properties of calcium signals at multiple intracellular scales – from microdomains to mesoscopic motifs and whole branches. Such analysis provides a template of calcium signaling patterns that can unequivocally put in relation to anatomical features and accounts for the richness of astrocytic dynamics documented in the literature.

T20-015C

Fibrinogen Regulates Astrocyte Scar Border Formation after Vascular Damage

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Scar-forming reactive astrocytes at the border of damaged neuronal tissue organize into a barrier surrounding the fibrotic lesion core, separating this central region of inflammation and fibrosis from healthy tissue. Lesion border astrocytes are essential for wound repair but interfere with neuronal regeneration. However, the mechanisms driving lesion border reactive astrocyte identity during CNS disease are unknown. Here we show that blood-derived fibrinogen is enriched at the interface of scar border-forming elongated astrocytes after cortical brain injury. In vivo systemic pharmacologic depletion of fibrinogen or inhibiting fibrinogen conversion into fibrin in mice reduces astrocyte activation, neuronal cell death and inflammation with no change in the spread of inflammation. Fibrinogen represses astrocyte polarization and migration by controlling connexin 30 expression in vitro. Fibrinogen depletion in mice resulted in the absence of elongated astrocytes and an altered extracellular matrix deposition at the lesion border. We propose that in CNS disease, fibrinogen serves as a critical trigger for scar border-forming astrocyte properties after cortical brain injury.

T20-016D

Birthdating of SOX9+ astrocytes during embryonic and postnatal development reveals their timed production from neural stem/progenitor cells in restricted cortical layers.

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Astrocytes are a highly heterogeneous population of cells with distinct gene expression profiles, phenotypes, and functions that reflect the varied environments in different regions of the central nervous system. To date, it is unknown whether distinct astrocyte subtypes are produced according to the temporal patterning of embryonic neural stem/progenitor cells (NSPCs) during development. We hypothesize that cortical astrocyte distribution is influenced by time of birth. To determine whether NSPCs give rise to distinct astrocyte subtypes that occupy different cortical layers according to developmental time, we electroporated embryonic NSPCs in the ventricular/subventricular zone (V-SVZ) with PiggyBac (PB)-CAG-EGFP at E15, E16, and E17 and sacrificed animals at P21. We electroporated postnatal NSPCs with a Cre-conditional PB-CAG-LSL-EGFP and PB-CAG-CreERT2 at P0 followed by tamoxifen injections from P1-7 and sacrificed at P21. Immunohistochemistry for the astrocyte lineage marker SOX9 was performed to identify EGFP+SOX9+ cortical astrocyte lineage cells. EGFP+SOX9+ astrocytes from E15- and E16-labelled NSPCs were mostly found in the upper layers, while EGFP+SOX9+ astrocytes from E17-labelled NSPCs were found in both lower and upper layers. In the early postnatal period, EGFP+SOX9+ astrocytes occupied mostly the lower layers at P21; very few EGFP+SOX9+ astrocytes were observed in the upper layers. Our results suggest that the distribution of SOX9+ cortical astrocytes may be influenced by their time of birth. Further analysis would determine whether other subsets of astrocytes exhibit similar or different cortical organization.

T20-017D

Protective microglial subset in development, aging and disease

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Microglial heterogeneity can reflect microenvironmental influences or intrinsic cellular differences. Recent transcriptomic studies describe microglial signatures, often relating them to a defined phenotype or subset. Many of these signatures show similar gene expression patterns, suggesting nomenclature redundancy. In this study, we aimed to address this issue for one particular subset of microglia and define it at a transcriptomic level.
We selected four studies based on their transcriptomic analyses of neonatal microglia. All four studies analyzed subsets with similar kinetics, morphology, markers, location and/or function, although isolated and named differently in each study. We compared the upregulated gene lists for each dataset and analysis using Venn diagrams highlighted 39 shared genes. We propose that these in fact represent a single unique microglial neonatal subset that is involved in primary myelination and characterized by a 39-gene signature.
We then selected four studies that generated transcriptomic datasets from microglial subsets in disease contexts (three different neurodegeneration models and one demyelination model). Choice of these subsets was again largely motivated by shared morphology, markers and function. Our comparison of upregulated gene lists followed by Venn diagram representation highlighted 89 shared genes. We propose that although these subsets were initially identified in different contexts and named differently, they represent a single unique microglia subset associated with disease protective functions, characterized by an 89-gene signature.
We subsequently cross-compared the two signatures we characterized and found that they overlapped by 22 genes, identifying a core signature for a microglial subset which expands in development and in some disease conditions. Analysis of these 22 proteins using the STRING database confirmed that this protein network exhibits significantly more biological connections than what is expected from 22 random proteins.
Our analyses indicate that microglia clusters identified by recent transcriptomic studies named in a variety of ways in fact represent a single microglia subset that plays important roles in development and protection of the CNS. We believe that these data will lead to unification of microglial nomenclature, which is a necessary step to avoid redundancy in future studies and will enable progress in our understanding of microglia biology.

T20-018D

IIIG9 inhibition in adult ependymal cells change adherent junction structure and induce cellular detachment

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Ependymal cells form a polarized cell epithelium with multiple apical cilia that line the ventricular surfaces and the spinal cord central canal. The normal differentiation of the ependymal cell inhibits hydrocephalus formation and allows a normal flow of CSF. In cancer, its normal polarization inhibits the formation of ependymoma-like tumors, for instance, supra-tentorial anaplastic ependymomas, highly aggressive in children. IIIG9 (PPP1R32) is a protein restricted to adult ependymal cells, located in the cilia, as well as in apical cytoplasm, with unknown function. In this work we have studied the expression and localization of IIIG9 in the adherent junctions (cadherin / b-catenin positive) of adult brain ependymal cells using confocal and transmission electron microscopy. Through IIIG9 *in vivo* loss-of-function studies, we reported the presence of ependymal denudation (single-dose injection experiments of inhibitory adenovirus), inducing the formation of ependymal cells with “balloon-like” morphology. These cells showed cadherins reduced expression (and/or delocalization) and cell death marker caspase 3 cleaved. Prior to aforementioned alterations, we observed “cilia rigidity” morphology (probably vibrational beating activity) and ventriculomegaly. Finally, performing continuous infusions of adenovirus for 14 days we observed a total cell denudation, and reactive parenchymal astrogliosis. Our data confirmed that IIIG9 is essential for the maintenance of adherent junctions of polarized ependymal cells. Eventually, altered levels of this protein in ependymal cell differentiation may increase ventricular pathologies, such as hydrocephalus or neoplastic transformation.

Acknowledgement
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T20-019D

Women neuroscientists disciples of Pío del Río-Hortega: the Cajal School spreads in Europe and South America

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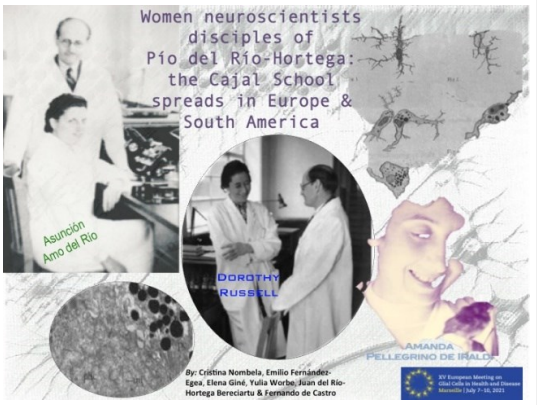
Pío del Rio-Hortega was not only the discoverer of the microglia and oligodendroglia but also possibly the most prolific mentor of all Santiago Ramón y Cajal´s disciples (Nobel awardee in Physiology or Medicine 1906 and considered as the father of modern Neuroscience). Among Río-Hortega´s mentees, three exceptional women are frequently forgotten, chronologically: 1) Pio's niece Asunción Amo del Río, technician specialized in the study of nervous system and specially neural tumours, who worked with Río-Hortega at Madrid, Paris and Oxford; 2-the distinguished British neuropathologist Dorothy Russell, who also worked with *Don* Pío at Oxford, where she completed her technical formation to become one of the leaders in the field of Neuropathology (in general), and brain tumours (in particular), after the death of Río-Hortega; and 3) Amalia Pellegrino de Iraldi, the last mentee in his career, at Buenos Aires, who develop most of her career as professor at the Universidad de Buenos Aires, after being formed with Eduardo De Robertis (she was maybe his most distinguished collaborator) and Arvid Carlsson (Nobel laureate in 2000), making fundamental contributions to the synaptic vesicles, cytoskeleton, and different Hortegian subjects. Here we introduce and discuss the research and contributions of these women at Río-Hortega´s laboratory and thereafter for better comprehension of the History and its frame. The present work completes the contribution of women neuroscientists that worked with Cajal and his main disciples of the Spanish Neurological School both in Spain (previous work) and in other countries (present work).



The women disciples of Pío del Río-Hortega Asunción Amo del Río (Spanish), Dr. Dorothy Russell (Australian British) and Prof. Amanda Pellegrino de Iraldi (Argenitinian) were formed by Pío del Río-Hortega and worked with him in different ethapes of his life.

T20-020D

Does the retinal photoreceptor composition influence Müller cell heterogeneity?

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Purpose: The human macula, key for accurate vision, is exceptionally prone to neurodegenerative processes.We aim to elucidate whether a functional heterogeneity of Müller cells, the major macroglia of the retina, may explain part of this macular susceptibility. To clarify the genetic basis of this heterogeneity, we generated and analyzed high quality proteomic data from cone- and rod-rich systems from human and mice.

Methods: Müller cells, microglia, vascular cells and retinal neurons from all cone R91W;Nrl$^{-/-}$ and R91W control mice as well as from human macular and peripheral samples were isolated by immunomagnetic separation and searched for differential protein expression by use of tandem mass spectrometry. Cell type and retinal region specificity of proteins were additionally tested by evaluating their expression in published scRNAseq datasets, while particularly promising candidates were subjected to increased scrutiny. To test the role of one of these, we generated CRISPR mediated KO lines of MIO-M1 cells, which are of Müller cell origin. A culture system based on acrylamide gels of varying compliances was used to measure changes in exerted shear stress, while cytoskeletal immunostainings coupled with a bioinformatics pipeline enabled the assessment of morphological features.

Results: We found significant differences in protein expression between predominantly cone- and rod-associated Müller cells in the human as well as the murine system, strengthening our hypothesis of functional Müller cell heterogeneity in the human retina. Indeed, some proteins showed a Müller cell-specific expression pattern in both the scRNAseq and our own proteomic data making these candidates especially promising for further research. *Eppk1*, though poorly understood,is thought to play a role in cytoskeleton/intermediate filament organization, which we were able to partly corroborate by showing its mislocalization in *Gfap/Vim*^{KO} retina. Furthermore, *Eppk1* knockout in MIO-M1 cells lead to a decrease in exerted shear stress as well as a change in size, shape, and filopodia characteristics.

Conclusion: We could show that the consistently different expression profile of some proteins in human and murine Müller glia yields candidates that may reveal functionally distinct cell subpopulations. Using various cell culture based techniques, we were able to lay the groundwork for the understanding of *Eppk1* function for the biophysical properties of Müller cells that might be of special importance for macular cells. Further studies in higher order model systems might improve the understanding of why the human macula is so sensitive to disease-associated changes and open up opportunities for the generation of novel therapies for pathologies like age-related macular degeneration.

T20-021E

Oligodendroglia heterogeneity in the post-mortem human CNS with region, age and sex

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Oligodendroglia are known to be heterogeneous in their developmental origin, morphology, propensity to myelinate and function (reviewed by Bøstrand & Williams 2021[1]). In recent years, studying their heterogeneity based on single cell transcriptomic data has led to a better understanding of the underlying changes in the CNS in the face of demyelinating and neuropsychiatric diseases. However, oligodendroglia heterogeneity between different CNS regions, sex groups and changes with ageing have not been studied in normal human control tissue, although it may be of interest: remyelination after demyelination has been shown to become less efficient with age[2] and women are more likely to suffer from the demyelinating disease multiple sclerosis; however, men usually suffer more severely from it[3]. Also, murine oligodendroglia isolated from the spinal cord produce longer and thicker myelin sheaths than those isolated from the brain[4]. As part of the Human Cell Atlas International Consortium, we defined oligodendrocyte heterogeneity over age, sex and CNS region by investigating the transcriptomic differences at a single nucleus level.

The present study included a total of 58 post-mortem human CNS samples of two different age groups: young adults (30 -45 years) and old adults (60 -75 years). For each of the 20 donors (ten male), three different CNS regions were analysed: motor cortex (BA4), cerebellum (CB) and cervical spinal cord (CSC). Nuclei were extracted

from the white matter of fresh-frozen tissue and used for single nuclei barcoding on the 10X Genomics (v.3) platform.

All expected cell types were present in our dataset (Figure 1). When focussing on oligodendroglia, we confirmed that they were transcriptionally heterogeneous and could be grouped into subcluster 'states', similar to our previous analyses[5]. We further found that at the transcriptional level regional differences outweighed age and sex effects. Most interestingly, we found differences in the oligodendrocyte precursor cell (OPC) population with the NELL1 expressing cluster OPC3 being highly enriched in BA4 and the PAX3 expressing cluster OPC1 being present almost exclusively in CB and CSC. Another interesting cluster was OL5 which is significantly enriched in CSC, shows a high expression of myelin genes such as PLP1 but also presents some overlap in its gene expression with the astrocyte population, for example by expressing SPARC.
In conclusion, we show that oligodendroglia are heterogeneous in human post-mortem control tissue and varies more with region than with sex and age group.

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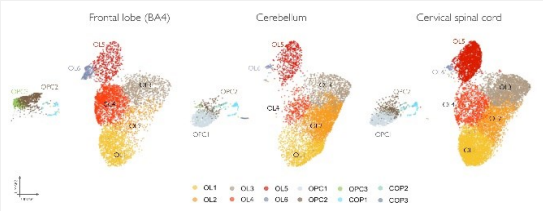

Figure 2: Oligodendroglia heterogeneity with CNS region. OPC = Oligodendrocyte precursor cell, COP =

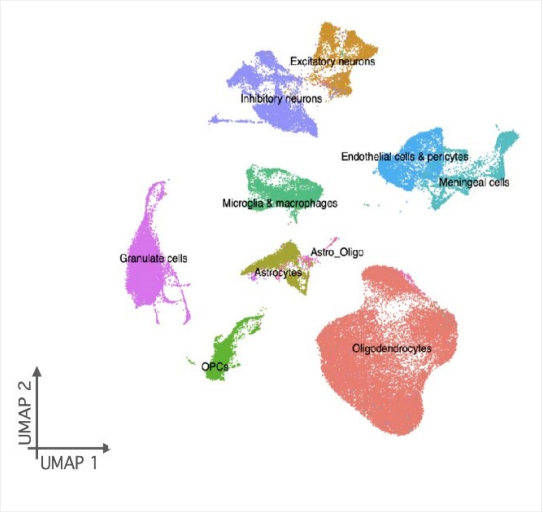

Figure 1: Complete dataset shows that all expected cell types are present.

T20-022E

Molecular and ultrastructural characterization of glial cells in the developing amphioxus: implications for the evolution of glia in chordates

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Glial cells have key roles in the development and homeostasis of metazoan nervous systems. However, while their involvement in the development and function in the nervous systems of vertebrates is increasingly well understood, much less is known about invertebrate glia. Consequently, the evolutionary history of glial cells remains elusive. Amphioxus, being the best living proxy for the last common ancestor of all chordates, provides a window on glial cells development and function at the transition between invertebrates and vertebrates. Here, we present an ultrastructural and molecular characterization of glial cells in the developing amphioxus. The combination of ultrastructural and gene expression data revealed that amphioxus glial lineages express genes typical of vertebrate astroglia and radial glia and that they segregate early in development, forming what appears to be a spatially separate cell proliferation zone positioned laterally, between the dorsal and ventral zones of neural cell proliferation. Our study provides strong evidence for the presence of vertebrate-type glial cells in amphioxus and highlights the importance of different progenitor cell pools for the development of the amphioxus nervous system.

There are implications also for our understanding of glial cells in a broader evolutionary context and insights into patterns of precursor cell deployment in the chordate nerve cord.

T20-023E

The impact of diurnal rhythmicity on microglia

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Diurnal rhythms are driven by sleep and circadian rhythmicity, both of which are important for physical and mental well-being, including the regulation of inflammatory processes.
As microglia play a crucial role in brain inflammatory processes, we investigated if microglial morphology, which closely correlates with function, is affected by the time of the day by sampling microglial cells throughout a 24h period.
We found a decrease in cell volume, territory, and other morphological features during the light period, which corresponds to the time of the day that mice spend mostly asleep, compared to the dark phase that mice spend mostly awake (N_{cells}=50-70, $p<0.05$, N_{mice}=4-7).
We proposed that these differences are at least partly mediated by sleep and therefore tested this assumption by manipulating sleep. Therefore we acutely sleep deprived (SD; 9 hours; gentle handling method) and sleep fragmented (SF; 14 days; fully automatic Sleep Fragmentation chamber, Lafayette, USA) male C57BL/6JR mice.
After the treatment, the animals were perfused and cortical microglia assessed via immunohistochemistry (Iba1, Synaptic Systems, Germany) and confocal z-stacks and analyzed via 3D reconstruction and tracing with a MatLab code (3DMorph, York et al., 2019). The results show that cortical microglia are affected by SD as well as SF: Whereas SD leads to an increase, SF leads to a decrease of the number of microglial branches (N_{cells} =13, $p<0.005$, N_{mice}=3). These changes of morphology did not equal the response to the immune stimulant lipopolysaccharide (i.p. injection; 5mg/kg) (N_{cells} =10, $p<0.005$, N_{mice}=4), which suggests that SD and SF lead to a different physiological function than the classical reactive state.
These findings show first evidence that daily rhythms and insufficient sleep regulate microglial morphology and, thereby, function.

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T20-025E

The expression and function of Kir4.1 in Schwann cells using Kir4.1-CreERT2 mice

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The molecular identity of terminal/perisynaptic Schwann cells (TPSCs) at the neuromuscular junction (NMJ) is unclear. We used mouse models and RNA-Seq to identify TPSC-specific genes. We crossed Wnt1-Cre mice,

which drive expression in all Schwann cells of the nerve, as well as TPSCs, to Ribotag mice, which express an epitope-tagged ribosomal protein, and dissected the motor endplate of the diaphragm and the sciatic nerve and compared transcriptomes. We found several genes more highly expressed in TPSC-enriched endplate samples. We pursued one candidate, KCNJ10, the gene encoding the inwardly-rectifying K+ channel Kir4.1. With an antibody, we observed robust expression in TPSCs at the NMJ and limited expression in axonal Schwann cells of the nerve. We made transgenic mice expressing inducible, estrogen receptor (ER)-fused Cre recombinase under the control of KCNJ10 regulatory elements. When we crossed this mouse to conditional tdTomato mice, and activated Cre with tamoxifen in young adult mice, we found that all TPSCs of the diaphragm expressed tdTomato. However, tdTomato was also expressed in L1 Cam+ non-myelinating Schwann cells of the sciatic nerve. In contrast, when we activated Cre at neonatal time periods (~P0-P3), we observed tdTomato in myelin protein zero (MPZ)+ myelinating Schwann cells as well some non-myelinating Schwann cells. These data suggest that Kir4.1 is expressed by either Schwann cell precursors or immature Schwann cells, then becomes restricted to non-myelinating Schwann cells after myelination. We also examined expression of tdTomato in the spinal cord as well as peripheral ganglia, glands and organs after tamoxifen administration at various timepoints. After young adult TMX injection, tdTomato was expressed by GFAP+ astrocytes and NG2+ oligodendrocyte precursor cells, S100+ satellite glial cells of sensory ganglia and adrenal gland, S100+ enteric glial cells, but not S100+ glia associated with sympathetic nerve innervating spleen. In order to investigate the role of Kir4.1 in Schwann cells, we first recorded membrane currents from freshly plated Schwann cells of neonatal sciatic nerve in response to voltage steps in the presence of different extracellular K+ concentrations and found that some Schwann cells exhibited inward rectification in response to a change from 5 to 10-15 mM K+. Finally, we investigated synaptic transmission in the diaphragm in the presence or absence of the specific Kir4.1 antagonist VU0134992 and observed transmission failure even at low frequencies of phrenic nerve stimulation. Together, these data suggest Kir4.1 is widely expressed by central and peripheral glial cells and plays a functional role in regulating neuromuscular transmission.

T21 | Neuromodulation by glia

T21-001A

Cortical astrocytes independently regulate sleep depth and duration via separate GPCR pathways

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Non-rapid eye movement (NREM) sleep, characterized by slow-wave electrophysiological activity, underlies several critical neural circuit functions, including learning and memory. However, NREM sleep is heterogeneous, varying spatially across the cerebral cortex, in both duration and depth. While these NREM sleep features are thought to be largely independently regulated, there is also evidence that they are mechanistically coupled. To investigate how cortical NREM sleep features are controlled, we examined the astrocytic network, comprising a cortex-wide syncytium that influences population-level neuronal activity. Performing two-photon imaging and simultaneous electrophysiology recordings, we quantified endogenous astrocyte activity over natural sleep and wake, then manipulated specific astrocytic G-protein-coupled receptor (GPCR) signaling pathways *in vivo*. We find that astrocytic Gi- and Gq-coupled GPCR signaling separately control NREM sleep depth and duration, respectively and that astrocytic signaling causes differential changes to sleep features in local and remote cortex. These data support a model in which the cortical astrocyte network serves as a hub for regulating distinct NREM sleep features.

T21-002A

Norrin modulates neuronal network communication and synaptic biology via a cortical astrocyte-subgroup signaling pathway

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Astrocytes are the most abundant cell type in the central nervous system (CNS) and are essential for brain function. As such, astrocytes play critical roles in ionic and glutamate balance, providing metabolic support to neurons, and regulating neuronal communication. Additionally, there is increasing evidence that implicates astrocytes in the proper development of the CNS. Astrocytic involvement in the maturation and maintenance of neuronal networks continues to be demonstrated and complemented by the identification of novel astrocyte-derived molecules that are critical to this process.

Our group recently identified a subset of astrocytes in the cerebral cortex that can be distinguished based on their expression of the alternative Wnt receptor ligand Norrin. Previously, Norrin has been extensively studied as a protein critical for vascular development in the retina and as a driver of the rare neurodevelopmental genetic disorder Norrie Disease (ND). However, characterization of Norrin's effects within the brain has been very limited in scope. This selective expression of Norrin in astrocytes within layer II/III and V of the adult cerebral cortex was found to influence neural network communication and synaptic structure. Specifically, adult Norrin-null (*Ndp*$^{-/-}$) animals had a significant decrease in synaptic length and density, that accompanied behavioral deficits. Additionally, *in vitro* experiments demonstrated that application of Norrin to primary cortical neuron cultures (PCNC) significantly increased firing frequency and neuron connectivity, as demonstrated by multi-electrode array (MEA) analysis. Subsequently, we have generated two mouse models of ND that carry the human equivalent of the V43E and H41X *NDP* mutations. Initial characterization of these mice has shown behavioral deficits that recapitulate the human disease and synaptic structural deficits. *In vitro* PCNC experiments have also demonstrated that Norrin modulates the expression of synaptic components. Collectively, these findings suggest that Norrin plays a significant modulatory role within the neural network and during synapse formation and function in the cerebral cortex.

T21-003A

Evidence for a central role of astrocyte α1A adrenoreceptor in the regulation of the excitatory/inhibitory balance and plasticity in the primary visual cortex.

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Noradrenaline (NA, also known as norepinephrine) is a major neuromodulator in the central nervous system (CNS). It is released from varicosities on neuronal efferents, which originate principally from the main noradrenergic nuclei of the brain - the Locus Coeruleus – and spread throughout the parenchyma. Noradrenaline is released in response to various stimuli and has complex physiological effects, in large part due to the wide diversity of noradrenergic receptors expressed in the brain, which trigger diverse signaling pathways. Virtually all CNS cell types express a number of different adrenoreceptors, meaning it is difficult to establish the precise contribution of a given cell type to any NA-mediated effect. Although previous *in vivo* work has found α1-NAR activation induces large increases in intracellular Ca^{2+} in cortical astrocytes, following the phasic release of NA during startling stimuli, a direct action of NA on astrocytic α1-NAR has not been established. To investigate this further, we decided to test a possible role for the α1**A**-NAR in astrocyte signaling, given it is the most highly expressed α1-NAR subtype in cortical astrocytes. Using an AAV-based shRNA knockdown strategy, we unambiguously show for the first time that direct activation of α1**A**-NAR can trigger intracellular Ca^{2+} increases in primary visual cortex astrocytes. Furthermore, we demonstrate that this response plays a critical role in modulation of the excitatory/inhibitory balance of cortical neuronal networks, and that induction of cortical long-term potentiation is dramatically altered in mice with reduced astrocytic α1**A**-NAR expression. Hence, our work reveals a critical role for astrocytes in mediating the neuromodulatory effects of NA in cortex.

T21-004A

Microglia depletion disrupts postnatal retinal development

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Precise neuronal wiring is the foundation of accurate nervous system function. The retina has a functionally and anatomically well-defined circuit, which is established during the first three weeks of postnatal development in mice. These developmental steps involve programmed cell death of retinal ganglion cells (RGCs), the formation of synaptic connections, and the fine-tuning of synaptic connectivity between bipolar cells and RGCs. Before synaptic refinement, RGCs respond similarly to all types of light stimulation (ON-OFF RGC). During maturation, their light responses are refined, such that they respond exclusively to increasing or decreasing light (ON or OFF RGCs, respectively). So far, the role of microglia is unclear in this circuit refinement, which then fundamentally determines visual signal processing.

Here, we investigated whether microglia are involved in these developmental changes. First, we observed that microglia frequently engulf RGC somas during early postnatal development, and consequently can strongly impact RGC density. When we depleted microglia from birth with PLX5622-containing chow, the adult retinas retained significantly more RGCs compared to control. In addition, we found that transcription factors, which are responsible for RGC terminal differentiation, were expressed by much fewer RGCs.

Next, we established a time-line for microglia interaction with RGC input-synapses using the Thy1-EGFP mouse model, in which RGCs are sparsely labeled with eGFP. When we stained the retinas for Iba1 and the excitatory postsynaptic marker PSD95, we found that microglia engulfed PSD95-eGFP co-localization puncta specifically after eye opening indicating active synapse-removal by the microglia.

Finally, to assess the functional consequences of microglia depletion on retinal circuitry, we recorded RGC light responses from acute whole-mount retina preparations using high-density CMOS multi-electrode array. In microglia-depleted retinas, the proportion of ON-OFF RGCs increased, while the ON RGCs decreased, compared to control retinas. Moreover, we detected a higher spontaneous RGC firing activity suggesting a more immature circuitry. In summary, our findings suggest that microglia play a significant role in the proper maturation of retinal structure and function.

T21-005A

Microglia contribution to neuronal network remodeling after paralysis onset

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Microglia are major mediators of experience-dependent synapse plasticity. Through this process, they contribute to neuronal network remodeling following sensory deprivation. Such neuronal network reorganization largely occurs in the brain after paralysis, a disability that dramatically alters the quality of life of those affected. To develop efficient

therapies, it is crucial to understand properly how the brain rearranges itself after loss of motor function. Previous studies focused mainly on macroscopic reorganization of cortical motor maps, however little is known about the cellular and molecular events that underlie this process. Microglia contribution, especially, remains unknown.
Here, we are investigating this question using a mouse model of unilateral hind limb paralysis. A single injection of botulinum toxin A (Botox, Allergan) was used to induce chemical denervation of the right calf muscles, therefore triggering a painless paralysis. Motor functions were evaluated through rotarod and tail suspension tests, revealing impairment as early as 1 day post-injection (dpi) and persisting at 3 and 7 dpi. After ensuring paralysis, we sought to identify brain areas affected by the motor deprivation. Neurons activated by a motor challenge were detected by staining the immediate early gene cFOS in whole brains cleared by the iDISCO$^+$ protocol and imaged by light sheet microscopy. The brain regions differentially activated in control and paralyzed animals are presently being investigated for potential differences in glutamatergic and GABAergic synapse density and in-depth microglia characterization.
Finally, because physiotherapy, the most common treatment for paralysis, also triggers network reorganization, we evaluated microglia contribution to its beneficial effects. Physiotherapy was mimicked by training control and paralyzed animals on the rotarod every 2 days, after feeding them a regular or a special diet inducing microglia-depletion. Although regular paralyzed animals could reach control scores by 7 dpi, microglia-depleted paralyzed mice failed to compensate for their poor performance as fast, reaching control levels only by 11 dpi. Further investigation of the cellular and molecular mechanisms are currently ongoing but this evidence already points to a crucial role for microglia in developing coping strategies following paralysis onset and therefore facilitating recovery.

T21-006B

Evaluation of the different AQP4 isoforms in the naïve olfactory-mediated behavior

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Aquaporin-4 (AQP4) water channel is expressed in two most abundant isoforms[1], called M1 and M23, of 32 and 30kDa respectively, which aggregates in the plasma membrane to form supramolecular assemblies, called orthogonal arrays of particles[2] (OAP). Recently, we reported the existence of two new extended AQP4 isoforms[3] (AQP4ex) generated by a translational readthrough mechanism, M1ex and M23ex,which are essential for perivascular astrocyte endefoot anchoring of AQP4 in the CNS. In the olfactory system AQP4 is expressed in supporting glial cells surrounding the receptor cells in the olfactory epithelium[4] (OE) and in the olfactory bulb[5] (OB). A previous study[6] reported that AQP4 knockout mice have impaired sense of smell, indicating a possible functional role of AQP4 in olfaction. The aim of our study was to evaluate the contribution of the different isoforms of AQP4 in the olfactory system by using two mouse models, AQP4ex-KO and AQP4M23-KO, respectively missing the AQP4ex and the M23 isoforms (OAP forming). Immunofluorescence experiments showed AQP4 expression in the basolateral membrane of supporting cells of the OE in the control mice. Interestingly, we found that AQP4ex was expressed, although at lower levels, in the same cellular compartment of the canonical (M1 and M23) AQP4 isoforms. As expected, the AQP4ex signal disappeared in the AQP4ex-KO mouse model, while no apparent changes were found for the canonical AQP4 isoforms. Also, when the most abundant isoform (M23) is absent, as in the AQP4M23-KO, most of the AQP4 signal was lost indicating the major contribution of the M23 canonical

isoform in the AQP4 plasma membrane assembly as it occurs in other CNS regions. In the OB, AQP4 and its extended isoforms revealed mainly an astrocytic perivascular expression pattern and a sparse reticular one in the glomerular layer. To elucidate the physiological role of AQP4ex and AQP4-M23 in the olfactory system, we performed a behavioral test, the “buried cookie test”, where food deprived mice were tested for four consecutive days and the latency to find the cookie was recorded. Surprisingly, AQP4M23-KO were faster in retrieving the cookie, thus showing greater olfactory abilities than control and AQP4ex-KO. No major olfactory defect was observed in AQP4ex-KO mice. These data suggest that AQP4 may play an important role in generating and maintaining the OE microenvironment necessary for olfactory sensitivity.

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T21-007B

Elucidating the role of BDNF/Astrocytic TrkB.T1 signaling on perisynaptic astrocyte process recruitment

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Astrocytes represent a morphologically complex central nervous system (CNS) cell type and they have emerged as critical players in both the development and physiology of the CNS. Their morphological complexity allows astrocytes to dynamically interact with neuronal synapses, where they establish and maintain synaptic connectivity by cradling synapses with peripheral astrocyte processes (PAPs). Astrocyte morphological maturation and the formation of synapses in neurons occur along a similar timeframe. However, the signaling mechanisms that draw a PAP to a synapse is largely unknown. In neurons, brain derived neurotrophic factor (BDNF) is a critical factor that promotes neuronal growth, survival, and synaptic refinement. RNA sequencing data from isolated astrocytes has demonstrated that astrocytes express high levels of the BDNF receptor, TrkB. Specifically, they predominantly express a truncated form of TrkB, TrkB.T1. Our recent work suggests that BDNF/TrkB.T1 signaling in astrocytes is an important signaling mechanism underlying astrocyte morphogenesis. Here, we examine if BDNF/TrkB.T1 signaling in astrocytes mediates the arrival of PAPs to developing synapses. *In vitro* co-cultures of wild type neurons with TrkB.T1 deficient astrocytes demonstrates that these morphologically immature astrocytes do not support normal synaptogenesis and function. *In vivo*, using TrkB.T1 knockout mice, immunohistochemistry and

puncta analysis of structurally formed synapses via pre- and post-synaptic (VGluT1/PSD95) co-localization suggests this receptor may mediate normal synapse formation. We further aim to manipulate the whisker barrel cortex to examine the arrival of PAPs to a synapse and the relevance of BDNF/TrkB.T1 signaling in this astrocyte experience-dependent structural plasticity. Alterations in synaptic function and development have been implicated in multiple neurodevelopmental disorders, but the majority of the research has only focused on the neuronal players. Our work will advance the understanding of the role of astrocytes in synaptic development and offer new groundwork for future therapeutic targeting.

T21-008B

Microglia control astrocyte isopotentiality and synaptic transmission in the mouse hippocampus

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Microglia and astrocytes are dynamic cells that closely associate with neurons and also actively survey and sculpt at the synapse. However, several likely mechanisms mediating microglia-neuron and microglia-astrocyte cross-talk have yet to be explored. Among these, a role for microglia in regulating the astrocyte syncytium, and the consequences for synaptic transmission, have not been evaluated. The cytoplasm of adjacent astrocytes is coupled through transmembrane gap junction channels, which permits the redistribution of ions, metabolic nutrients and signaling molecules in support of neuronal function. This continuous astrocyte network constantly equalizes the membrane potential of individual astrocytes, a property known as 'syncytial isopotentiality', and is required for extracellular K+ and neurotransmitter clearance. Here, we used pharmacological loss-of-function and gain-of function experiments to test the role of microglia in the astrocyte syncytial network and synaptic transmission. Using in situ electrophysiological recordings, we show that microglia depletion weakens the astrocyte syncytium and synaptic transmission in the young adult mouse hippocampus. Conversely, elevating microglia activity using systemic lipopolysaccharide strengthened synaptic transmission. Further mechanistic experiments indicate that microglial activity scales synaptic transmission and the expression of post-synaptic proteins. This study reveals a critical role for microglia as essential regulators of astrocyte physiology and synaptic transmission. This work also provides the basis for future studies exploring microglial control of astrocytes and synapses in various physiological and pathological contexts.

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T21-009B

Norepinephrine links astrocyte calcium activity to changes in cortical state

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The pattern of electrical activity in the neocortex fluctuates over time, even without external stimuli, in response to endogenous neuromodulators such as norepinephrine[1] (NE). In the visual cortex, these fluctuations in cortical state have been shown to modulate sensory processing and perception[2], but the mechanisms regulating cortical state

are not fully understood. Using *in vivo* two-photon calcium (Ca^{2+}) imaging in mouse visual cortex we describe that astrocytes, a cell type that can potently modulate cortical state, are driven by ongoing fluctuations in NE, even without large behavioral changes. In combination with *in vivo* electrophysiology, we show that NE-induced astrocyte Ca^{2+} activity is linked to neuronal synchrony locally and cortical state globally. Surprisingly, we find that the relationship between astrocyte Ca^{2+} and global cortical state does not require signaling from nearby neurons. Further, perturbing astrocyte sensitivity to NE causally ties astrocytes to the regulation of cortical state. This regulation distinctly affects both arousal-associated cortical state changes, and unexpectedly, visual stimulus-evoked changes in cortical state. Therefore, we demonstrate that NE signaling through astrocytes acts as a distinct neuromodulatory pathway, providing a new mechanism for the regulation of cortical state.

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T21-010B

Application of PLGA nanoparticles to enhance the action of Duloxetineon microglia in neuropathic pain.

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Duloxetine (DLX) is a selective serotonin and noradrenaline reuptake inhibitor (SNRI) used for treatment of pain, but it has been reported to show side effects in 10–20% of patients. Its analgesic efficacy in central pain is putatively related to its influence on descending inhibitory neuronal pathways. However, DLX can also affect the activation of microglia. This study was performed to investigate whether PLGA nanoparticles (NPs), which are expected to enhance targeting to microglia, can improve the analgesic efficacy and limit the side effects of DLX.PLGA NPs encapsulating a low dose of DLX (DLX NPs) were synthesized, characterized and their localization was determined. The analgesic and anti-inflammatory effects of DLX NPs were evaluated in a spinal-nerve-ligation (SNL)-induced neuropathic pain model.The analgesic effect of DLX lasted for only a few hours and disappeared within 1 day. However, DLX NPs alleviated mechanical allodynia, and this effect was maintained for 1 week. DLX NPs were localized to the spinal microglia and suppressed microglial activation, phosphorylation of p38/NF-κB-mediated pathways and the production of inflammatory cytokines in the spinal dorsal horn of SNL rats. We demonstrated that PLGA NPs encapsulating a low dose of DLX can provide a prolonged analgesic effect by

enhanced targeting of microglia. Our observations imply that the sustained release of DLX from NPs targeting microglia provides drug repositioning with a prolonged analgesic effect while reducing the potential side effects of abuse and overdose.

T21-011C

Astrocytic CD44 deletion in the dentate gyrus influences epileptogenesis.

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Epilepsy is one of the most common chronic neurological disorders, affecting up to 1% of the world's population. The current lack of effective pharmacotherapies is due to our limited understanding of the cellular mechanisms that underlie seizure activity. While it is widely accepted that epilepsy is caused by a combination of neuronal and astrocytic dysfunction, their specific correlation pathways in the brain remain poorly studied. Astrocytic processes are closely associated with synapses, enwrapping and interacting with dendritic spines and synaptic terminals. Our main focus is put on CD44 protein, a transmembrane receptor for hyaluronan, highly expressed in astrocytes in the central nervous system, which was reported to modulate synaptic plasticity. To study the CD44-depended astrocyte-neuron relationship in epileptogenesis, the kainic acid (KA) model of temporal lobe epilepsy (TLE) was used, where mice undergo intrahippocampal KA injections provoking *status epilepticus* and further recurrent seizures. Whereas wild-type (WT) mice injected with KA show similar to human neuropathological and electroencephalographic (EEG) TLE features, those with a previously activated conditional CD44 knock-out in astrocytic cells in the dentate gyrus develop a different seizure pattern. Their EEG recordings show the seizures they experience are more frequent than those in the control group, but their intensity is much lower. While WT mice experience more whole body clonic/clonic-tonic attacks, CD44 knock-out mice typically immobilize or do not show any behavioral symptoms of a seizure at all. These results indicate astrocytic CD44 plays a crucial role in epileptogenesis and seizure development and may provide a possible alternative pathway for future drug discovery.

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T21-012C

Astroglial mitochondrial calcium determines synaptic integration

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Astrocytes exert key metabolic, structural and protective functions. Over the past few years, novel features of these cells have emerged pointing to their direct participation in the so-called tripartite synapse, thereby modulating information processing and behavior. Very little is known about the intracellular astroglial mechanisms required to exert these functions, but it is now clear that calcium dynamics at different subcellular astroglial microdomains are key functional elements of the tripartite synapse. Here we show that the activation of astrocyte mitochondrial-associated CB1 receptors (mtCB1) determines intracellular calcium dynamics and lateral synaptic regulation through specific mechanisms that regulate the activity of the mitochondrial calcium uniporter (MCU) channel. Physiologically, mtCB1-dependent mitochondrial calcium uptake determines the precise dynamics of cytosolic calcium events in astrocytes upon endocannabinoid mobilization. Accordingly, electrophysiological recordings in hippocampal slices showed that genetic exclusion of specific astroglial mtCB1 receptors or MCU inhibition blocks lateral synaptic potentiation, a key example of astrocyte-dependent integration of distant synapses activity. Altogether, these data reveal an unforeseen link between astroglial mtCB1 and the regulation of brain network functions.

T21-013C

Oligodendrocyte Precursor Cells Sculpt the Visual System by Regulating Axon Remodeling

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The central nervous system comprises a large number of oligodendrocyte precursor cells (OPCs) that do not differentiate to form new myelin. The role of this cell population for nervous system function apart from giving rise to myelinated oligodendrocytes is largely unclear. Here, we show that the zebrafish optic tectum contains OPCs interspersed with retinotectal connections in regions devoid of myelin by the time a functional visual system is present. The processes of OPCs extend throughout the tectal neuropil, where retinal ganglion cell (RGC) axons frequently retract after contact with these processes. Early ablation of OPCs using nitroreductase and olig2 morpholino lead to formation of errorneous RGC axon branches reaching outside the tectal neuropil, as well as enlarged arbour sizes of individual RGC axons. Targeted OPC ablation using two-photon lasers, as well as global genetic OPC ablation at later stages during the maturation phase of retinotectal connectivity, disrupts axonal remodeling. As a consequence, late OPC ablation impaired prey capture behaviour and reduced visual acuity in optomotor response tests by showing increased response failure to stimuli of high-spatial frequency. Together, our findings demonstrate a non-canonical role of OPCs in regulating axonal remodeling, with consequences for neural circuity and animal behavior.

T21-014C

Optimisation and validation of a system X_c^- functional assay in cultured astrocytes and in nervous tissue samples

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In many neurological diseases, glutamatergic homeostasis was shown to be disrupted and this correlates with an alteration in the expression and/or the functionality of high affinity glutamate transporters (EAATs) in astrocytes. While many studies have already highlighted the importance of EAATs, there is a growing interest in the cystine/glutamate exchanger, also designated as the system X_c^-. Mainly expressed in glial cells, more specifically in microglia and astrocytes, its dysfunction has been documented in many neurological diseases such as Parkinson's disease, amyotrophic lateral sclerosis, epilepsy, or glioma.

System X_c^- is composed of two different subunits: the 4F2 heavy chain and the xCT light chain. While 4F2 is common to numerous carriers, xCT is responsible for the substrate specificity and transport activity. Under physiological conditions, system X_c^- operates along with the concentration gradient of its substrates, exchanging extracellular cystine for intracellular glutamate. By doing so, it provides the cells with the rate-limiting amino acid for glutathione synthesis and reinforces glutamate signalisation. Hence, it is considered as the major source of extracellular glutamate in the central nervous system. As for EAATs, tight regulation of its expression and activity is essential for the control of the excitatory synaptic activity.

While many studies report on the characterisation of xCT regulation by RT-qPCR or by Western blot using some few commercially available antibodies, the properties of this exchanger remain poorly examined in functional studies. Indeed, the documented use of sulphur-radiolabelled cystine in uptake assays shows several drawbacks including the short radioactive half-life and the high price of sulphur-radiolabelled compounds.Therefore, we have optimized a method using tritiated glutamate as a substrate for a reversed transport. The uptake assay was validated in primary cultured astrocytes, in diverse cell lines as well as in fresh nervous tissue samples obtained after homogenisation and centrifugation in isotonic conditions. Working in buffers containing defined concentrations of sodium, allowed us to distinguish the uptake supported by system X_c^- or by EAATs, as confirmed by using selective pharmacological inhibitors. The specificity was further demonstrated in samples from transgenic mice lacking xCT or in cell lines where xCT expression was induced or silenced. Our protocol appears as a robust and cost-efficient solution to investigate the functional roles of this exchanger in physiological and pathological conditions. It also provides a reliable tool for the screening and characterisation of new system X_c^- inhibitors which have been frequently cited as valuable drugs for nervous disorders.

T21-015C

Deciphering the Central Amygdala Oxytocin-Induced Astrocytic and Neuronal Intracellular Activity

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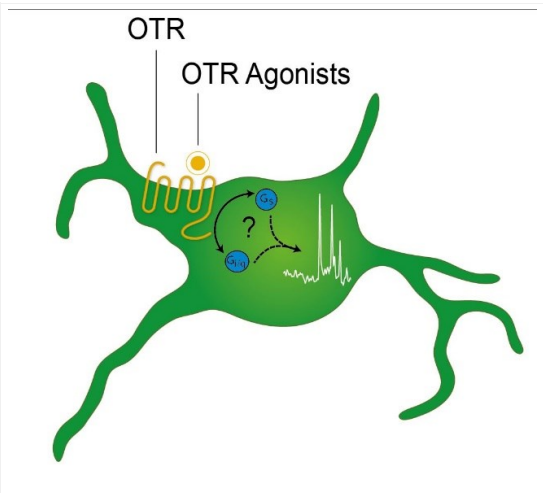
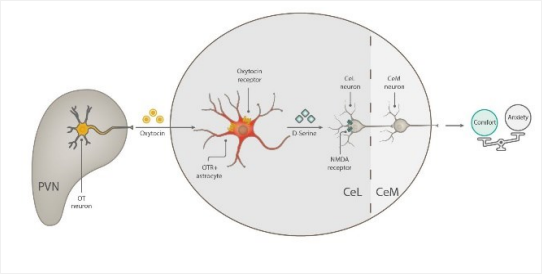
Oxytocin is a hypothalamic neuropeptide with a wide range of functions. From its crucial involvement in delivery to its roles in maternal behaviour, the functions of oxytocin have been studied for decades. The implication of this peptide have recently been highlighted in modulating neuronal networks responsible for complex behaviours, such as pain control, or anxiety modulation. However, oxytocin effects on glia have been left behind. Therefore, the actual dogma is that oxytocin, like other neuropeptides, acts exclusively via neuronal receptors in the central nervous system. To investigate the effects of oxytocin on astrocytes, we focused on the amygdala, were the role of oxytocin have been well characterized. Using patch clamp recording and calcium imaging, we have shown that astrocytes respond to an endogenous oxytocin release prior to neurons. Moreover, blocking or stimulating those glial cells can respectively decrease or mimic the effects of oxytocin on neuronal activity. At a behavioural level, these manipulations of amygdalar astrocytic activity respectively abolish or mimic the oxytocin effects on anxiety and place preference (Fig.1).

Our previous results have shown that the removal of astrocytic oxytocin receptor (OTR) abolish the effects of oxytocin in the central amygdala. However, around 70% of neurons express OTR in this structure. This counterintuitive fact brings us to question the role of neuronal receptors in the effect of oxytocin. To elucidate this point, we aimed to evaluate whether amygdala neurons and astrocytes OTR are recruited upon different concentrations of oxytocin. Knowing that OTR can be coupled to both $G\alpha_{i/o}$ and $G\alpha_{q}$ pathway, such differential recruitment may reflect the variety of OTR intracellular couplings. Hence, we took advantage of recently developed biased agonists to decipher the intracellular pathway(s) triggered by specific OTR stimulation in either neurons or astrocytes (Fig.2).

By unveiling this astro-neuronal circuit, this work aims at highlighting that neuropeptide can modulate neuronal networks *via* its action on astrocytes' calcium activity.



What is the intracellular path recruited during oxytocin-induced astrocyte activity?



Oxytocin stimulate amygdalar astrocytes to modulate the local microcircuit, promoting positive emoti
PVN: ParaVentricular Nuclei, CeL & CeM: CentroLateral & CentroMedian Amygdalar nuclei, OT: Oxytocin, OTR: Oxytocin Receptor.

T21-016C

RAGE activation in astrocytes contributes to neuronal-astrocytic communication through glutamate release

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Receptor for advanced glycation end products (RAGE) is a multiligand receptor involved in a variety of signalling pathways. In the brain, RAGE is known to be involved in neuroinflammation and the pathogenesis of different neurodegenerative disorders, including Alzheimer's disease; but also regulates many physiological processes such as neuronal differentiation. Besides AGES, formed by the glycation of proteins, lipids or nucleic acids, many other ligands can activate RAGE, including beta-amyloid. Synthetic RAGE fragments can also bind the receptor and be used to understand the mechanism of RAGE activation/inhibition. Here, we have used a synthetic fragment (residues 60-76) from the extracellular region of the receptor, which protects neurons against beta-amyloid toxicity (Volpina 2018, Kamynina 2018) to study its cellular effects in primary co-cultures of neurons and astrocytes.

We show that application of the 60-76 RAGE fragment leads to cytosolic calcium signals almost exclusively in neurons. These signals were caused by the fragment-induced RAGE activation, since they could be prevented by blocking RAGE with specific antagonists/antibodies. Interestingly, in these cultures RAGE was expressed in both neurons and astrocytes, but it was localized in the surface of the plasma membrane predominantly in astrocytes. We show calcium influx in neurons in response to RAGE fragment was mediated by neuronal glutamate receptors (NMDA and AMPA), since it could be eliminated when pre-incubating the cells with their antagonists MK801 and CNQX. Transfection of the cultures with the glutamate sensor iGluSnFR showed that the 60-76 RAGE fragment induced peaks of glutamate release in astrocytes (but not in neurons) that led to the observed calcium signals in the neighbouring neurons. Fusion of glutamatergic vesicles in astrocytes in response to the RAGE fragment was further confirmed with the astrocytic-targeted EGFP-VGLUT2 construct.

These results depict a mechanism by which RAGE activation can contribute to the communication between neurons and astrocytes in physiology and pathology.

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T22 | Psychiatric diseases

T22-001A

Chronic social stress leads to altered cortico-limbic oligodendrocyte and myelination status in adult mice.

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Stress-related psychiatric illnesses including major depressive disorder (MDD) and post-traumatic stress disorder (PTSD) often present with altered resting state functional connectivity (rsFC) in the anterior cingulate cortex-amygdala (ACC-AMY) network. This potentially underlies some major symptoms including increased reactivity to, reduced control of, and impaired extinction learning about aversive events. Whilst the responsible cellular pathologies remain to be identified, post-mortem studies report alterations of the oligodendrocyte lineage (OL) and myelin content in the ACC and AMY of MDD patients. Given the essential role of myelin in action potential propagation within/between brain regions, OL/myelin changes could impact on ACC-AMY network FC, resulting in aversion processing dysfunctions.

To investigate the causal inter-relationships between these factors, relevant animal models are essential. In rodents, the medial prefrontal cortex - mPFC: prelimbic cortex (PrL) and infralimbic cortex (IL) – exhibits some structural and functional analogy with the primate ACC. In male mice, 15-day chronic social stress (CSS) leads to altered mPFC-AMY rsFC and to changes in aversion processing comparable with the above clinical symptoms. Also, CSS downregulates the expression of OL- and axon-myelin unit-related genes in mPFC and AMY. The aims of the present study were to investigate CSS effects on OL lineage turnover and on myelin, in mPFC and AMY.

Adult male C57BL/6 CSS mice (n=14) remained distally exposed to aggressive resident mice for 15 days and underwent brief daily attack without wounding; control mice (CON, n=12) were handled daily. The animals had *ad libitum* access to 0.2mg/mL 5-ethynyl-2'-deoxyuridine (EdU) in drinking water. Half of the CSS/CON mice were perfused-fixated on day 16; the remaining mice on day 31. Tissue was labelled for EdU and OL markers to assess OL turnover, and for MBP and NF200 to assess myelin and axonal content.

In mPFC, CSS had no effect on OL lineage density or turnover. CSS led to increased MBP integrated density at day 31 specifically ($p<0.004$). In a separate cohort, ultrastructural quantification identified a tendency to increased myelin thickness (g-ratio $p<0.07$; thickness (µm) $p=0.1$) and myelinated fibre density ($p=0.05$) at day 16. In AMY, CSS led to an overall reduction in $NG2^+$ cell proliferation ($p=0.03$) and an increase in $CC1^+$ cells density at day 31 ($p<0.02$). It had no effect on MBP integrated density ($p>0.5$).

Thus, mouse chronic social stress without physical wounding alters OL and myelin status in mPFC and AMY, suggesting that these glial processes could contribute to the disrupted ACC-AMY network and aversion processing that pertain in this model.

Acknowledgement

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We thank Prof. Leda Dimou and her team for the helpful and stimulating scientific discussion.

T22-002A

Contrasting morphometric signatures of microglia from hyper- and hypofunctional dopaminergic pathways in an animal model of Schizophrenia

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Microglia are immune modulators that exert vital functions during neurodevelopment. The immune competence supports the ability to screen newborn synapses and to promote its maturation or elimination, according to the functional state. Any disturbance of these functions during critical periods of life has been associated with neurodevelopmental diseases.

Several risk factors along life, from gestational period until adolescence, have been implicated in Schizophrenia (SCZ), contributing for disease development and progression. SCZ patients can manifest a panoply of symptoms that includes positive (delusions and hallucinations), negative symptoms (self-neglect, loss of motivation and social withdrawal), as well as cognitive deficits, biologically associated with hyper- (positive) and hypofunctional (negative) dopaminergic pathways. We hypothesize that an imbalance in maturation/elimination functions of microglia in critical periods of life may underlie the dichotomic dopaminergic pathways described in SCZ. Notably, during brain development, microglia undergo a process of morphologic differentiation and we here propose that a pathogenic long-term alteration of morphology could be involved in the inappropriate functioning of dopaminergic pathways.

We used the methylazoxymethanol acetate (MAM, gestational day 17) rodent model of SCZ that resembles some negative symptoms (social withdrawal). Male and female offspring were subjected to neurodevelopmental tests and, at postnatal day (PND) 30, to cognitive and social behavioral tests. Then, brains were collected for microglia morphology characterization in the *Nucleus accumbens* (NAc) and prefrontal cortex (PFC), key brain regions in the dichotomic pathways aforementioned.

MAM males and females presented a transient delay in maternal odor discrimination that suggests an emotional impairment already in early development. Concordantly, at adolescence, despite the absence of cognitive deficits, MAM males and females displayed social deficits, ultimately demonstrating an impairment of social memory and predilection for novel experiences. Regarding microglia cytoarchitecture, despite the absence of changes in PFC, MAM males presented an atrophy of microglia at NAc, demonstrating that MAM males show distinct microglia morphologic signatures depending on the brain region and pathway involved.

These SCZ animal model can be used as a suitable tool to better explore the mechanisms underlying the negative symptomology in SCZ. Furthermore, at adolescence, microglia present distinct morphologic features that are coincident with the dichotomic dopaminergic pathways in SCZ only in males, which might suggest a dual involvement of these cells in SCZ genesis, an effect that is sex-dependent and should be further explored.

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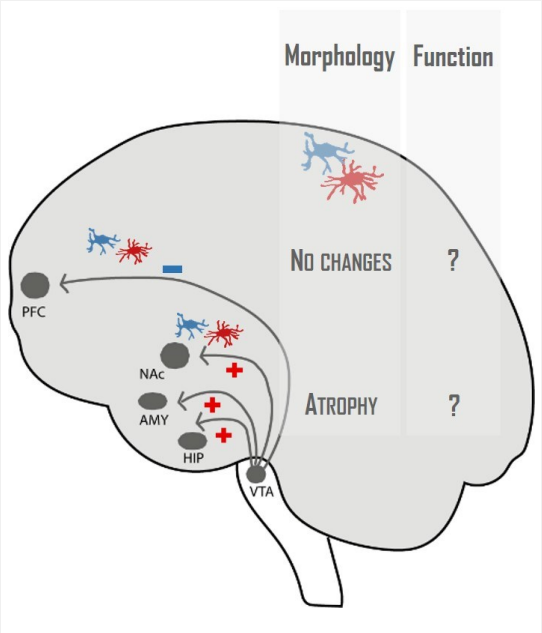
The authors declare no conflicts of interest.



Microglia cytoarchitecture in hyper- and hypofunctional pathways described in Schizophrenia
Distinct microglia cytoarchitecture (atrophy versus no changes) in hyper- (positive symptoms) and hypofunctional (negative symptoms) dopaminergic pathways well characterized in Schizophrenia pathophisiology

T22-003A

An *in vitro* stress model of primary oligodendrocytes from anxious and non-anxious inbred mouse strains

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Anxiety disorders are the most prevalent psychiatric illnesses that greatly deteriorate the quality of life. Predisposition to anxiety disorders involves a combination of genetic and environmental factors, such as psychosocial stress. We recently identified myelin plasticity as a major brain response to chronic social defeat stress in mice. Critically, myelin remodeling varied between stress resilient and susceptible animals, was brain region-specific, and was affected by the genetic background. To investigate the molecular and cellular mechanisms of myelin plasticity in a controlled environment, we set up an *in vitro* stress model consisting of primary oligodendrocytes (OLs) from C57BL/6NCrl (B6; innately non-anxious and mostly stress resilient) and DBA/2NCrl (D2; innately anxious and mostly stress susceptible) mouse strains. In this model, oligodendrocyte precursor cells (OPCs) are physically isolated after 10 days in a mixed glial cell culture, resulting in an enriched culture where OLs represented ~60% of cells. OLs expressed myelin-related genes and a complex arborization after 3 days. After 6 days, cells were fully mature and produced myelin. Ongoing efforts aim to establish the effect of acute and chronic exposure to corticosterone, the major stress hormone in mice, on proliferation, differentiation and cell death of OLs obtained from the two strains. As oxidative stress has also been observed in anxiety disorders, the same parameters will be investigated after exposing the cells to H_2O_2. This project aims to identify the specific mechanisms of stress-induced myelin plasticity and to contribute to the development of novel therapeutic targets to promote stress resilience mechanisms in individuals suffering from anxiety disorders.

T22-004A

Astrocytic calcium elevations in the basolateral amygdala during fear and extinction

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Impairments in fear extinction are thought to be central to the psychopathology of posttraumatic stress disorders. Although astrocytes are shown to be increasingly important for learning and memory, the role of astrocytes in fear extinction remains largely unknown. Here, we sought to investigate how changes in calcium elevations in astrocytes are associated with, or would impact cued fear extinction. To this end we utilized fiber photometry to monitor astrocytic Ca^{2+} activity in basolateral amygdala (BA) in behaving mice. We found that prominent Ca^{2+} transients in astrocytes were associated with foot shock during fear learning and were detected in response to the tone presentation during extinction learning, recent and remote memory tests. Notably, with the progression of extinction training, we saw a gradual decline in tone-associated responses in wild type mice. However, when we record astrocytic Ca^{2+} activity in the BA of inbred mouse strain with impaired fear extinction – this suppression was absent. Instead we observed larger Ca^{2+} transients during extinction. In previous in vitro studies, it had been demonstrated that activation of astrocytic cannabinoid type 1 receptors (CB1R) lead to intracellular Ca^{2+} elevations, triggering release of gliotransmitters and modulating synaptic transmission and plasticity. Therefore, we next asked how the deletion of astroglia CB1R would affect Ca^{2+} responses in astrocytes during fear extinction and how it would impact fear extinction memory. First, we demonstrated that fear extinction, but not fear learning, is facilitated in mice with both brain-wide and BA-specific deletion of astrocytic CB1R. Second, we found a decrease in astrocytic Ca^{2+} transients in CB1R deficient mice when compared to the wild type controls. Remarkably, these changes were evident when we recorded Ca^{2+} activity with membrane tethered, but not cytosolic form of genetically encoded Ca2+ indicators, underlying importance of astrocytic microdomain in CB1R mediated signaling. Finally, we

found that a reduction in Ca^{2+} signaling in BA astrocytes by overexpressing the plasma membrane Ca^{2+}-ATPase (PMCA) resulted in the facilitation of fear extinction learning and memory formation. Altogether, our data suggests that Ca^{2+} responses in astrocytes are tightly correlated with freezing level/fear state: large Ca^{2+} transients were detected during fear learning and in mice with impaired fear extinction, whereas augmented fear extinction was observed in mouse models with decreased astrocytic Ca^{2+} level due to deletion of CB1R or overexpression of PMCA in astrocytes. Future studies will investigate how astrocytes, and astrocytic CB1R, are implicated in shaping neuronal networks in the amygdala during fear extinction learning.

Acknowledgement
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T22-005A

Chronic Stress Induces Microglial-Mediated Inflammatory Responses and Compromises the Oligodendroglial & Neuronal Homeostasis Leading to Depression

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Background Depression is a chronic debilitating illness affecting yearly 350 million people worldwide. Although the mechanisms underlying depression are still not defined, it has been suggested that inflammation may mediate depression by promoting glial and neuronal dysfunction. Microglia, as the brain's resident innate immune cells, can be activated by both immune (i.e. infection) and nonimmune challenges (i.e. psychosocial stress), and contribute to the regulation of neuropsychiatric disorders[1]. The aim of the study is to characterize the inflammatory responses during chronic social stress and determine their effects on the homeostasis of oligodendroglia (OL) and neurons in a depression mouse model.

Methods The Repeated Social Defeat Stress (RSDS) paradigm (10 days) was utilized to study the early (5 days) and post RSDS stages (10+5 days and 10+15 days) in 8–12-week-old male C57BL/6J, *CX3CR1*-GFP^{+}, *CSPG4*-EGFP^{+} mice. To investigate a requisite role for microglia in mediating depression, the mice were fed at the beginning of the RSDS paradigm either with control chow or chow containing the Csf1R inhibitor PLX5622 [~90% microglial elimination]. Behavioral tasks (BH1 & BH2) were performed to categorize the defeated mice to susceptible (S; depressive-like) and resilient (R; non-depressive) to stress groups. The study focuses on the MDD-affected prefrontal cortex (mPFC) area (Fig.1a).

Results Inflammation was observed at D15, depicted by the presence of activated Iba1 cells [reactive morphology, expressing CD68, inflammatory markers (CD86, TSPO, CD206, iNOS) and inflammatory cytokines] in the S groups. Microglial proliferation (BrdU) and peripheral macrophage recruitment (CD11b^{+}CD45^{high}) were also examined after chronic stress, revealing a significant increase in the S groups. Interestingly, the microglial activation significantly affected the phagocytosis of myelin (CNP, MBP) and synaptic elements (PSD95, Syn-I). Remarkably, microglial ablation in the socially defeated mice protected them against the psychological effects of chronic stress and prevented depressive-like behavior post RSDS (Fig.1b). Significant changes were also identified in the OL progeny as significant reductions of OL progenitor cells (OPCs; PDGFRα and *CSPG4*-EGFP^{+}) and morphological alterations were observed in mPFC in the S groups. The OPC mitotic capacity (BrdU, Ki67) and progeny progression were examined during- and post-RSDS, revealing time-dependent changes in the proliferation and increased OPC differentiation (O4, GST-pi) in the S groups.

Conclusions Our data suggest that chronic stress induces microglial activation and recruitment, dynamically compromising neuronal and OL homeostasis, leading to depressive-like behavior in mice (Fig.2).

Acknowledgement

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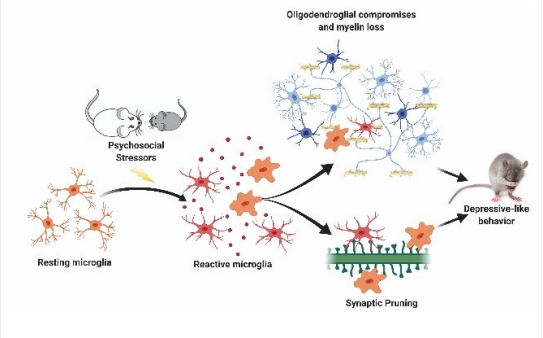

Figure 2. Graphical outline of the study
Chronic stress induces microglial activation and inflammatory responses, swiftly compromising the oligodendroglial and neuronal homeostasis, leading to depressive-like behavior.

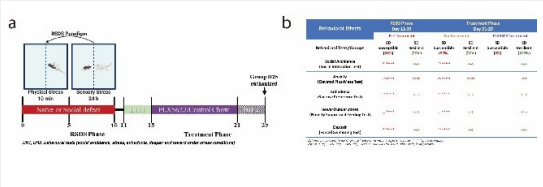

Figure 1. CSF1 receptor inhibitor rescues chronic-stress induced depressive-like phenotype
CSF1 receptor inhibitor PLX5622 post the RSDS paradigm was sufficient to exert antidepressant like effects, signifying the importance and correlative relationship of microglial-induced inflammation and depression-like behavior.

T22-006A

Altered calcium signaling in astrocytes of Fragile X Syndrome mouse model

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Fragile X syndrome (FXS) is caused by the silencing of the fragile X mental retardation protein (FMRP). Patients with FXS are often also diagnosed with autism spectrum disorder and exhibit additional symptoms which may include intellectual disability, seizures, anxiety, and sensory hypersensitivity. FMRP, encoded by the *fmr1* gene, is known to regulate protein expression via interactions with mRNA or protein. Despite extensive characterization of FMRP function, particularly in neurons, the effects of FMRP silencing on astrocyte physiology remain understudied. We have found increased expression of the metabotropic purinergic receptor P2Y1 in the cortex of Fmr1 knockout mice. P2Y1 receptor is a key regulator of astrocytic calcium transients and notably has been found to mediate aberrant calcium signaling in models of neurological diseases. In accordance, we have found enhanced spontaneous and ATP-induced somaticcalcium signaling in astrocytes in acute brain slices from *fmr1* knockout mice. Pretreatment with MRS2179, a P2Y1 receptor antagonist, normalized the amplitude and frequency of calcium events in slices from *fmr1* knockout mice. We also observed a smaller enhancement of ATP-induced calcium signaling in mice in which FMRP is selectively deleted in astrocytes. This suggests that the mechanisms underlying elevatedcalcium signaling in the knockout are both intrinsic and extrinsic, and that altered astrocytic function may contribute to the altered structural and functional properties of neurons in FXS. In vivo imaging of astrocyte calcium signaling and behavioral analyses of global and astrocyte conditional fmr1 KO mice are ongoing.

T22-007B

Microglial spatio-temporal heterogeneity in a perinatal inflammation mouse model – Link to Autism-like phenotypes

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A general consensus regarding neurodevelopmental disorders including Autism Spectrum Disorder (ASD) is that they originate from early development defects in brain formation, leading to altered neuronal circuitry responsible for the pathological behavior. Despite studies on genetic implication in ASDs, a causal relationship between genomic alteration and ASD has been difficult to explain in many cases, suggesting environmental factors might be involved. In fact, preterm birth is often linked to the occurrence of inflammation and preterm infants have a ten times higher risk of developing ADS-like symptoms than infants born at term. Moreover, some clinicial studies reported ongoing neuroinflammation processes in different brain regions in autistic infants including frontal cortex, hippocampus and cerebellum. The major relay of the environmental response in the brain, including inflammatory

responses, is microglia cells (MG), the brain resident macrophages that continuously survey their local environment. Moreover, during development microglia play a critical role during the synaptic pruning to contribute to the formation of the mature cerebral connectivity network. In an inflammatory context, MG are activated and participated to the local release of pro-inflammatory cytokines. Our hypothesis is, therefore, that an exposition to perinatal inflammation impacts on neurodevelopmental disorder symptoms leading to ASD. Using a mouse model of perinatal inflammation induced by IL1b injection between post natal day (P)1-5, this project demonstrates that i) there is region specific inflammation between frontal cortex, hippocampus and cerebellum determinate; ii) an impact of microglial activation on the synaptic pruning at P15 and a modulation of connectivity by UltraFast Doppler at P40; and iii) an impact of the perinatal inflammation on the onset of ASD-like phenotypes at different developmental stages by UltraSonic Vocalization (P2 and P8), Nest Odor preferency test (P8) and an adapted three-chamber test (P40). This innovative project has as objective to identify potential diagnosis markers to facilitate an early detection of ASD in premature infants based on inflammatory indicators.

T22-008B

Immune Phenotypes of Oligodendroglial-Lineage Cells in MDD and in Response to Chronic Stress-Induced Microglial Inflammation

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Background Major Depressive Disorder (MDD) is a complex and heterogenous psychiatric disorder affecting more than 300 million people worldwide. Though the mechanisms underlying MDD are not fully understood, a subset of patients can manifest chronic neuroinflammatory responses. Brain-imaging studies in MDD patients have provided evidence of white matter reductions, and such changes are hypothesized to lead to disruption of Oligodendroglial-Lineage cells (OLN) homeostatic mechanisms, resulting in destabilization of emotional/cognitive circuitry. The goal of the study is to investigate whether these histopathological alterations are linked with chronic inflammation, using single-nucleus transcriptomics of MDD patients and in a depression mouse model.**Methods** *Bioinformatics:* Raw counts were downloaded from GEO (GSE144136), and expression objects created using Seurat. OLN were subsetted, clustered (using UMAP), and annotated based on their expression of early and late OLN markers. Pseudotime trajectory analysis was conducted using Moncle3.*In vivo:* The Repeated Social Defeat Stress (RSDS) paradigm (10 days) was used to model depressive-like behavior in 8–12-week-old male *CX3CR1*-GFP^{+} and *CSPG4*-EGFP^{+} mice. Behavioral tasks (BH) were used to stratify the defeated mice into susceptible (S; depressive-like) and resilient (R; non-depressive) to stress groups. The study focuses on the MDD-affected prefrontal cortex (mPFC) area (Fig. 1).

Results Separate clustering of MDD and Control samples yielded four similar groups labeled based on their gene expression as: OPCs, Committed OPCs, Immature Oligodendrocytes (OLs), and Mature OLs. One MDD-specific cluster was also obtained and labeled as Immune OLs (ImOL), given its expression of immune genes, such as: *ARGHAP24, ADAM28, LPAR6, C3, CD74,* and *P2RY12*. Further analysis of the ImOL cluster revealed expression of markers associated with later phases of OLN progeny. Finally, a predictive model of pseudotime trajectory revealed alterations in the progression of OLN progeny in MDD samples. We sought to identify this novel subset of ImOL in the RSDS model of depression. MHCII, C3 and P2RY12 were examined in conjunction with OLN markers (CSPG4, O4, CNP), with significant changes observed in S groups. Interestingly, microglial phagocytosis of myelin elements (*CX3CR1*-GFP, MHCII, CNP) was significantly increased in S groups post RSDS, suggesting that the ImOL populations may function as potential recruiters of innate immunity.

Conclusions Together, our computational data in MDD patients and depression mouse model, show that OLN can swiftly respond to chronic stress, and adopt an immune-like phenotype, potentially contributing to the white matter disturbances observed in MDD.

Acknowledgement

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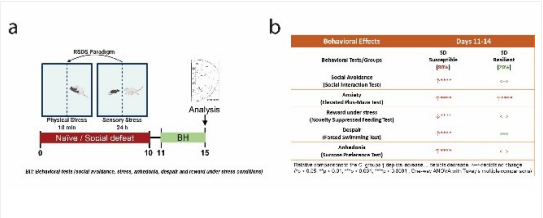

Figure 1. Graphic outline of RSDS paradigm and respective behavioral assessment

T22-009B

Role of adult hippocampal neurogenesis in the antidepressant effects of lactate

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Astrocytes are key players in energy metabolism and glutamate transport. In addition, growing evidence suggests that astrocytes are involved in the pathophysiology and treatment of depression. For instance, SSRIs stimulate lactate release from cortical astrocytes. Recently, we showed that acute lactate administration increased lactate concentration in the hippocampus and reduced immobility in the forced swim test (Carrard et al, Mol Psychiatry 2018). Further investigation of the antidepressant behavioral effects of lactate revealed that chronic administration of lactate improved depressive-like behavior in the corticosterone model of depression and in the open-space forced swim test (Carrard et al, Mol Psychiatry 2018). The antidepressant effects of lactate are associated with changes in the expression of specific target genes among which Hes5 and p11 are involved in adult hippocampal neurogenesis. These findings led us to investigate the role of adult hippocampal neurogenesis in the antidepressant effects of lactate.

The involvement of adult hippocampal neurogenesis in the antidepressant effects of lactate was examined in the corticosterone model of depression. We found that chronic peripheral injection of lactate counteracted the decreased neural progenitor proliferation and survival induced by corticosterone treatment. In contrast, chronic administration of pyruvate did not produce antidepressant-like effects and did not prevent the inhibition of neural progenitor proliferation and survival induced by chronic corticosterone injection. Importantly, depletion of adult hippocampal neurogenesis by administration of the antimitotic drug temozolomide suppressed the antidepressant-like effects of lactate on behavioral despair and anhedonia in animals chronically treated with corticosterone.

In vitro studies on hippocampal stem cell cultures revealed that corticosterone decreased cell proliferation and increased ROS production. Consistent with our *in vivo* observations, lactate but not pyruvate suppressed the effect of corticosterone on ROS production and partially counteracted the effect of corticosterone on stem cell

proliferation. Similarly to lactate, NADH prevented ROS production elicited by corticosterone and partially reversed the inhibition of stem cell proliferation induced by corticosterone. Therefore, our data suggest that conversion of lactate to pyruvate with the concomitant production of NADH is necessary for the neurogenic and antidepressant effects of lactate. Collectively, these data emphasize the importance of adult hippocampal neurogenesis in the antidepressant effects of lactate.

References

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T22-010B

Characteristics of sialylation and ST6Gal I in serum and brain in mouse model of depression

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Depression has been interpreted as a neuroimmune disease, in which activated microglia is the main source of inflammatory cytokines in the brain. Numerous evidence suggest that aberrant sialylation, a common protein glycosylation often accompanied by changes in sialyltransferase activity, can affect immune regulation in the brain. It is found that the expression of β-galactoside α2,6-sialytranferase I（ST6Gal I）, catalyzing the α2,6-linked sialic residue to the terminal N-linked glycans, was increased in osteoarthritis. However, changes of sialylation and ST6Gal I expression in inflammatory responses in depression remains unknown yet. In the present study, we established a mouse model of depression by intraperitoneal injection of lipopolysaccharide (LPS,1mg/kg), and 24h later, LPS-injected mice showed the depression-like behaviors assessed by open field test, elevated plus-maze, forced swimming test and tail suspension test. Sialylation in N-Glycans analyzed by mass spectrometry (MS) and lectin blotting were extremely up-regulated in serum and brain of depressive mice. Meanwhile, the expression ofα2,6-linked sialic acid, ST6Gal I and proinflammatory cytokines (TNF-α, IL-1β, IL-6) detected by quantitative PCR and ELISA were all increased. Further, similar changes ofα2,6-linked sialic acid, ST6Gal I and cytokine expression were observed in primary microglia and BV_2 cells. The data suggest that enhanced sialylation and ST6Gal I expression in microglia and serum could contribute to brain and peripherial inflammatory responses in depression. The present study provides a novel aspect for further investigation of inflammatory mechanisms of depression.

T22-012B

Transcriptomic responses of microglia to a chronic, unpredictable, mild stress in the prefrontal cortex and hippocampus in a murine model of depression

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Depression, according to WHO, will be the leading cause of disease burden by 2030, and 30% of the patients remain unresponsive to current treatments. Stressful life events and environmental stressors increase the risk of depression and alter gene expression that may lead to aberrant neuronal functions in regions implicated in disease pathogenesis. Microglia, are brain resident immune cells, that control brain homeostasis, monitor the functional state of synapses and influence neuronal plasticity. **Aims**: We sought to determine if and how microglia respond to chronic stress inducing depression-like behavior in mice. We employed a chronic unpredictable mild stress (CUMS) in mice to emulate the effects of chronic stress in the brain and explore its effects on behavior, microglial morphology and functions. We studied if minocycline, an inflammation reducing agent, could mitigate stress-induced changes in microglia. **Methods & Results**: We used cell sorting of CDb11+ cells from prefrontal cortices and hippocampi of control, stressed and treated animals and RNAseq, we found different transcriptomic responses in each group of animals. **Conclusions**: CUMS emulates chronic stress effects behaviorally and provokes transcriptomic and morphological changes in microglial cells.

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T23 | Glia – glia interaction

T23-002E

Astrocyte-targeted overexpression of both IL-6 and IL-10 modifies postnatal white matter-associated microglia and affects primary developmental myelination

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Microglia are the main immune cells of the CNS, originated in the yolk salk in embryonic stages. During postnatal development, microglia is involved in homeostatic and developmental functions, such as synaptic pruning or neurogenesis. Recent studies showed that a specific ameboid microglial population arising in the corpus callosum (CC) during development affects myelination. Indeed, the activation and number of postnatal microglia has been related to structural alterations in the mature brain, that result in developmental diseases. Previous results from our group showed that transgenic mice overexpressing either anti-inflammatory interleukin (IL)-10 or pro-inflammatory IL-6 under the GFAP promoter (GFAP-IL10Tg, GFAP-IL6Tg) contained more microglia/macrophages in grey matter areas during development. Thus, the main objective of this study was to determine whether microglia located in white matter (CC) was also increased and their phenotype modified during development, and if it affected the postnatal myelination. With this aim, brain samples of GFAP-IL10Tg and GFAP-IL6Tg mice and their respective WT were obtained from postnatal day 0 (P0) to P28 and adults. Immunohistochemistry was performed to determine microglia phenotype and number (Iba1, Pu.1, CD11c, and Galectin-3) as well as myelination (MBP, Olig2). Our results indicate that neonatal ameboid microglia in WT increased progressively from P0 to P7, and at P14 changed to ramified morphology. In GFAP-IL10Tg and GFAP-IL6Tg, more ameboid microglia with higher Iba1 expression was found in the CC at P5 and P7, respectively. Later, at P14 and P21, GFAP-IL10Tg maintained high microglia numbers, opposed to WT and GFAP-IL6Tg, where microglia decreased progressively until the adult. Determination of myelination showed few MBP+ oligodendrocytes (OL) in WT at P5, and myelination rose quickly at P14 until completion in the adult. While GFAP-IL6Tg increased its myelination earlier (P7) in comparison to WT, GFAP-IL10Tg showed more MBP staining than WT only at P14. Despite differences in MBP, the OL population (Olig2+) in both Tg mice was not modified compared to WT. Since differences in microglial populations were related to changes in myelination, we explored Galectin-3 and CD11c expression in postnatal microglia, both molecules described as promoters of OL maturation and myelination. While Gal-3 expression was enclosed to the first ameboid stages and its expression did not differ in Tg mice, CD11c expression was restricted to P5 and P7 and was slightly modified in GFAP-IL10Tg. In conclusion, our study indicates that IL-6 or IL-10 overexpression induced modifications in white matter microglia during postnatal stages that may affect myelination. Further studies are needed to decipher the mechanisms involved.

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T23-003E

Characterization of Ca^{2+} signals in spinal glial cells using transgenic mice with cell-specific GCaMP expression

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Glial cells (astrocytes, oligodendrocytes and their precursor cells as well as microglia) exhibit Ca^{2+} changes as responses from the communication network they are embedded in. So far, the complexity, i.e. the spatio-temporal pattern of Ca^{2+} signals is poorly understood.

Here, we focused on the characterization ofspontaneous Ca^{2+} transients in white and gray matter tracts of the mouse. For that purpose, we appliedtwo-photon laser-scanning microscopy (2P-LSM) and recorded Ca^{2+} changes in the mouse spinal cord *in vivo* but also in *ex vivo* preparations. Spinal glial cells could be imaged *in vivo* after a laminectomy and positioning of a glass window. Acute vibratome cross sections of the dissected spinal cord were used as *ex vivo* preparation. In our study, we took advantage of different transgenic mouse lines (TgH(CX_3CR_1-CreERT2), TgN(hGFAP-CreERT2), TgH(NG2-CreERT2) and TgN(PLP-CreERT2)), expressing the genetically encoded Ca^{2+} indicator GCaMP3 induced by tamoxifen injection. Each field-of view, containing several Ca^{2+} signals was analyzed by the custom-made, automatic MATLAB based software suite MSparkles which provided cell-intrinsic Ca^{2+} signal properties such as amplitude, duration and signal frequency. In order to compare astrocytes, oligodendrocyte lineage cells and microglia, the individual signals were automatically detected. Distinct cell type-specific patterns of Ca^{2+} transients could be identified *in vivo* as well as *ex vivo.* In general, the signals were characterized by a high heterogeneity. The heterogeneity was quite cell-selective, and independent from the selected imaging mode: *ex vivo* or *in vivo*. The signal dynamics, like average and maximum area as well as peak and duration were the main parameter to discriminate individual glial cell types.

Our study revealed a high diversity of glial Ca^{2+} signaling in the spinal cord, demonstrated by 2P-LSM imaging, but independent from *in vivo* or *ex vivo* preparations.

T23-004E

How mad can microglia become when you kill their astrocytes. The possibility of shifting active microglia phenotype after fluorocitrate-induced astrocyte death - a pharmacological perspective.

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Introduction: Recently, we have shown that prolonged fluorocitrate (FC) infusion into the *substantia nigra* (SN) induced astrocyte death and caused strong, local microglia activation. Here, we present preliminary test results of pharmacological tools designed to modulate or suppress such microglia activation *in vivo*.

Methods: Prolonged intracerebral FC infusion with osmotic minipumps was used to induce astrocyte death and consequent, strong microglia activation in the SN. Modulatory drugs were administered twice a day for 7 days (*ip*.): minocycline (MC) to suppress microglia activation and fasudil (FAS), a potent Rho-kinase inhibitor, to shift microglia polarization from a cytotoxic towards more cytoprotective phenotype. Immunohistochemical staining of microglia general marker IBA-1 was used for the Scholl analysis of microglia morphology, while fluorescent staining – IBA-1 and polarization markers (CD86/CD206) – was used for colocalization.
Results: FC significantly induced microglia activation. Strong increment of area covered with IBA-1(+) cells was observed independently of any pharmacological treatment. The number of microglial branch intersections after FC administration was strongly reduced, which confirmed the morphological change into a less ramified shape. MC, but not FAS, reduced thisactivation parameter. Colocalization staining confirmed that FC increased IBA-1 marker expression but neither MC nor FAS administration affected it significantly. Furthermore, astrocyte death increased the expression of a pro-inflammatory marker CD86, and MC reduced this effect, while FAS showed a similar, yet slighter trend. Anti-inflammatory microglia polarization marker CD206 did not show any significant changes due to the treatments.
Conclusion: Local, chronic infusion of the astrocytic toxin, FC, results in strong activation and proliferation of IBA-1(+) cells in the SN. Additional pharmacological treatment with MC decreases but not eliminates microglia activation. FAS treatment appears to show analogical tendencies to MC, but to a lesser degree. The presented data show that local astroglia depletion results in strong microglia activation with a potentially cytotoxic, proinflammatory pattern, and only this phenotype development can be slightly prevented by pharmacological modulators such as MC or FAS, with no influence on a microglia activation level. Changing the activated microglia phenotype instead of its deactivation could be a better, more functional therapeutic approach, however, the strength of activation factor forces adjustment in the choice of pharmacological treatment.

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T23-005E

Time-of-Day-Dependent Expression of Astrocytic Connexins in the Hippocampus

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Gap junctions represent direct cell-to-cell communications that allow trafficking of small molecules (< 1.5 kDa) including ions, neurotransmitters, and metabolites such as glucose between the adjacent cells, and are therefore essential for intercellular metabolic and electric coupling. A gap junction is formed of Connexin (Cx) proteins arranged as two opposed leakless hexameric hemichannels (connexons). The astroglial connexins Cx30 and Cx43 contribute to many important CNS functions including cognitive behavior, motor function and regulation of the sleep-wake cycle. Our previous results show a time-of-day-dependent fluctuation of Cx30 and Cx43 protein and mRNA expression in the suprachiasmatic nucleus, in mice exposed to changing light conditions. We concluded that astroglial Cx30 and Cx43 play a role in rhythm stability and re-entrainment under challenging conditions. In

hippocampus, astroglial connexins has been linked to various functions including adult neurogenesis and cognition. However, it is not known yet whether the Cx30 and Cx43 show a time-of day dependent fluctuation in the hippocampus.

In this study, we analyze the expression of the astroglial connexins Cx30 and Cx43 under different light conditions including LD 12:12, constant darkness (DD) and constant light (LL) in the dorsal hippocampus in male C57Bl/6J mice using immunohistochemistry. Under LD 12:12, Cx30- and Cx43-immunoreaction (Ir) show a time-of-day-dependent expression with a higher level during the light/inactive phase. This suggests that light and or inactivity has a positive effect on the expression of astrocytic connexins. However, under DD conditions, the Cx30-Ir was higher during the early subjective night/activity phase while Cx43-Ir was not rhythmic. This suggests that in absence of rhythmic light cues, activity has a positive effect on Cx30-Ir only. Under LL, both astroglial connexins Cx30 and Cx43 were equally arrhythmic, consistent with arrhythmic behavior in spontaneous locomotor activity. In summary, the astroglial connexins Cx30 and Cx43 in the dorsal hippocampus show a rhythmic expression driven by the light/dark cycle rather than by the circadian system.