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ABSTRACT BOOKLET

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Parallel session abstracts

1.1. Molecular Neurodegeneration

Elucidating the Role of Brain Senescence and Telomere Attrition in Alzheimer's Disease Tauopathy

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Background Neurodegenerative diseases are the most common age-related pathologies, including Alzheimer's disease (AD). Recent studies suggest that telomere attrition, the main trigger of cellular senescence, might contribute to brain dysfunction and neurodegeneration with age (Forero et al., 2016). Senescent brain cells have been found in AD patients (Musi et al., 2018), and studies in a transgenic mouse model of tauopathy have shown that the elimination of senescent cells led to an improvement in tau-related neurodegeneration and cognitive decline (Bussian et al., 2018). However, little is known about the exact connection between senescent brain cells and neurodegenerative disorders on a cellular and molecular level. Here, we studied how brain senescence relates to the onset and progression of AD-related tau pathology.

Method We used a mouse model of senescence (TercKO), which is deficient for the RNA component of the telomerase, and presents telomere attrition and premature ageing with increasing generations (Blasco et al., 1997). We crossed it with the tauopathy mouse model Tau P301S (PS19), which carries the P301S mutant form of the human microtubule-associated protein tau gene (MAPT), exhibiting tau pathology features observed in AD (Yoshiyama et al., 2007). By taking advantage of primary cultures, brain sections and brain extracts, and using biochemistry and molecular biology techniques, we examined the expression of tau neuropathological features in a senescent context. Furthermore, we evaluated the changes in classical and novel markers of senescence in a neurodegenerative context.

Result We observed that telomere attrition induces several known senescence markers (i.e., senescence-associated beta-galactosidase or SA-β-gal activity, upregulation of interleukins IL-1β and IL-6, and cell cycle regulator p16) and this exacerbates tau phosphorylation in primary neurons and hippocampal tissue. Additionally, our results suggest that the senescent context might enhance tau-induced neurodegeneration.

Conclusion Our work indicates that senescence might be an upstream regulator of tau pathology, aggravating it and triggering tau-related neurodegeneration. Our results further suggest that TercKO mice could be a useful animal model to study the pathological role of age-associated brain senescence. Elucidating this process in more detail could lead to the identification of suitable intervention targets for AD treatment.

Keywords: Brain Senescence, Alzheimer's Disease, Tau Pathology

Investigating dysregulated miRNAs in Alzheimer's disease for biomarker discovery and brain-serum correlation

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Background The utilization of blood microRNA (miRNA) has been proposed as a potential biomarker for Alzheimer's Disease (AD) owing to its cost-effectiveness and less invasive nature. However, a challenge emerges as the role of circulating miRNAs in AD pathology remains exclusive. Hence, it is advantageous to uncover the correlation between circulating miRNAs and dysregulated miRNAs in the AD brain. Furthermore, investigating the origin of blood miRNAs is crucial.

Methods In this study, we identified differentially expressed miRNAs in AD across three brain regions, namely the gyrus cinguli, gyrus temporalis superior, and Brodmann a6. The combination of feature selection and differential expression analysis was used. All the data was obtained by sequencing. The mRNA transcriptomics of these brain tissues were also utilized to explore the regulatory association between miRNAs and their gene targets. Subsequently, we analyzed the levels of these miRNAs in cerebrospinal fluid (CSF) and serum samples obtained from the same individuals with AD. Additionally, we examined the EXOmotifs, which are sorting sequences dictating the secretion of miRNAs in small extracellular vesicles, in these dysregulated miRNAs. Furthermore, we proposed the potential of miRNAs that are dysregulated in the AD brain and correlated in serum samples as biomarkers for AD. We validated this proposition in both our own dataset, which consists of 9 AD cases and 10 controls, and an external dataset GSE120584 comprising 305 AD cases and 149 controls.

Results Overall, 39 miRNAs were identified as dysregulated in the AD brain, of which 22 were previously recognized as differentially expressed in AD. Among these, miR-132-3p, miR-132-5p, miR-212-5p, miR-412-5p, and miR-30a-3p consistently showed dysregulation across all three brain regions. Interestingly, the gene targets of miR-132-3p expressed in different brain regions exhibited enrichment in distinct biological processes including metabolic process, neurogenesis etc. To align with our focus on easily obtainable specimens, we delved into the functions of dysregulated miRNAs that exhibit high correlations ($r > 0.5$ or $r < -0.5$) when detected in serum. Specifically, miR-1306-5p, miR-132-3p, miR-30a-3p, miR-501-3p, miR-15a-5p, miR-323b-3p, miR-628-3p, and miR-409-3p was included. The regulated gene targets of these miRNAs showed enrichment in AD-relevant pathways such as PI3K-Akt signaling and inflammation-related pathways. Out of the 39 dysregulated miRNAs, 13 were detected in CSF/serum samples, with 30.8% and 46.2% respectively containing EXOmotifs. While in our 8 dysregulated and highly correlated miRNAs, 50% contained EXOmotifs, suggesting that circulating miRNAs tend to be EXOmotif-containing. Moreover, highly correlated miRNAs between the brain and serum are more likely to be EXOmotif-containing, hinting at the possibility that these circulating miRNAs may originate from brain cells or indicate a potential connection between brain miRNAs and circulating miRNAs. We postulated that the 8 miRNAs, which were dysregulated in AD and highly correlated across brain and serum, could serve as potential biomarkers for AD. Using GSE120584, we trained a logistic regression model of the 8-miRNA panel with 75% samples and validated it with 25% samples, achieving an AUC of 0.77. In our own dataset, the 8-miRNAs achieved an AUC score of 0.9, showing great potential as biomarkers.

Keywords: Alzheimer’s disease; microRNAs; biomarkers

Outbreak of tauopathy and associated features in the olfactory and central nervous systems of PS19 mice

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Background: Clinical data indicate that olfactory function impairment (OI) serves as an early indicator of neurodegenerative diseases (ND), including Alzheimer's disease (AD). OI tends to manifest early in 90% of AD patients. Neurofibrillary tangles (NFTs) – Tau lesions – have been observed in the entorhinal cortex and olfactory bulbs from AD patients, knowing that these regions play a role in olfactory information processing. Our hypothesis is that typical lesions of ND could appear early in regions of the olfactory system. Here, we address the question of the occurrence of tauopathy in regions such as the olfactory epithelium (OE), olfactory bulb (OB), piriform cortex, entorhinal cortex, and hippocampus of transgenic mice.

Methods: We used heterozygous PS19 mice, described as a tauopathy mouse model by Yoshiyama et al. (2007). These transgenic mice express the human tau protein (1N4R) carrying the P301S mutation. They develop NFTs-like inclusions in the cortex and hippocampus around six months, leading to progressive neurodegeneration at eight months. Mouse samples were collected from heterozygous PS19 and wild-type (WT) mice. Staining methods such as DAB staining or immunofluorescence were used to demonstrate the expression of hyperphosphorylated human tau protein (hTau). We investigated specific regions of the olfactory system such as the olfactory epithelium and bulbs to correlate with data obtained in specific CNS regions (e.g. piriform and entorhinal cortex, hippocampal formation). Specific methods such as decalcification were implemented to preserve the olfactory epithelium structure. Western blots were performed to monitor tau expression levels and tau phosphorylation pattern.

Results: We observed exclusive detection of hyperphosphorylated human tau protein (Ser202, Thr205) in PS19 mice sections, while the endogenous murine tau protein was expressed both in WT and PS19 mice. The localization and expression patterns of these proteins varied according to the region (OE or CNS subregions), the age (3, 6 or 9 months), and the genotype of the mice (WT or heterozygous PS19). In the OE, phospho-Tau was expressed in the olfactory neurons within the middle stratum as early as 3 months. In the olfactory bulbs, pTau was mainly expressed in the olfactory nerve layer. Tau lesions were found in the piriform and entorhinal cortex from 6 months, along with the CA3 region and dentate gyrus in the hippocampus. All these data suggest that pTau may appear early in the olfactory epithelium and subsequently spread from the peripheral to the central nervous system following neuroanatomical pathways, like the olfactory or perforant pathways. These findings highlight: (i) the prognostic potential of the olfactory epithelium region in the diagnosis of tauopathies and (ii) its likely contribution to the pathology progression in the CNS.

Keywords: Tauopathy, Olfactory system, NFTs

The PDE4D inhibitor RICE improves spatial memory performance and neuroplasticity-related processes

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Alzheimer's disease is the most common cause of dementia worldwide. While phosphodiesterase type 4 (PDE4) inhibitors have shown promising results for memory enhancement in affected individuals, their clinical use is hampered due to severe side effects such as nausea and vomiting. However, the PDE4 family consists of many different subtypes and isoforms. The PDE4D subtype is involved in neuronal functioning and memory formation, and selective inhibition of PDE4D has been shown to provide cognitive benefits without adverse effects. This study introduces RICE, a newly developed PDE4D inhibitor (patent pending WO2022253959A1). We evaluated the potential of RICE in neuroplasticity and memory processes. We report that RICE shows selectivity for PDE4D2 and PDE4D7, indicating more selective PDE4D isoform targeting compared to previous PDE4D inhibitors. We found RICE to enhance the outgrowth of neurites in vitro. In vivo experiments indicate that RICE is non-emetic and has excellent brain penetration. Furthermore, RICE improves spatial memory performance in the object location task in healthy C57BL/6J mice after low-dose subcutaneous and intranasal administration. In conclusion, we argue that RICE holds promise as a therapeutic strategy for Alzheimer's disease, offering a selective and effective approach to address cognitive decline.

Keywords: phosphodiesterase 4D, object location task, intranasal

1.2. Environment, bran & behaviour

Complement-Mediated Control of Glutamate Transmission in Cortical Tripartite Synapses of Mice with Experimental Autoimmune Encephalomyelitis

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In recent years, the participation of the complement in the course of the disorder in multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), was identified. It was observed that the complement influences blood-brain barrier permeability, favouring the infiltration of peripheral lymphocytes and circulating autoantibodies. Furthermore, the complement shows a principal role in innate immunity within the central nervous system (CNS). Particularly, C1q and C3 proteins were identified as contributors to microgliosis, astrocytosis, and synaptic engulfment, key processes in MS and EAE progression. We recently inquired about novel function of complement in regulating glutamate release from nerve endings in the cortex, with implications for normal excitatory transmission and pathological excitotoxicity. Now we asked if this phenomenon in the cortex of EAE mice revealed correlations with synaptic and astrocytic abnormalities during the acute disease stage. This study characterizes intricate interactions within the quad-partite synapse in the cortex of EAE mice, highlighting complementinduced modulation of glutamate release and its implications for synaptic communication. Specifically, we focused on the distribution of C1q and C3 proteins, along with the role of complement as a promoter of glutamate release, in purified nerve endings (synaptosomes) and astrocytic processes (gliosomes) isolated from the cortex of EAE mice at the acute stage. In the cortex of EAE mice at 21 ± 1 days post immunization (d.p.i.), cortical synaptosomes and gliosomes exhibited no noticeable changes in viability or apoptosis compared to controls. However, a significant increase in C1q and C3 proteins was observed together with microgliosis (quantified as CD11b density), astrocytosis (quantified as GFAP density), and synaptic derangements (measured as SNAP25 and PSD95 density). Moreover, the glutamate exocytosis was significantly reduced in EAE cortical synaptosomes and gliosomes. Interestingly, the complement-evoked releasing activity was almost halved in synaptosomes but increased in gliosomes. Furthermore, we noticed that EAAT2 and EAAT1, glutamate transporters responsible for reuptaking and regulating extracellular glutamate levels in the CNS, behave differently between synaptosomes and gliosomes. The EAAT2 density in EAE synaptosomes was significantly lower than in controls, while EAAT1 density in gliosomes increased. This reflects the complement's opposing modulation of glutamate transmission at synaptic terminals and astrocytic processes. Therefore, the activity of complement at glutamatergic cortical synapses is altered in EAE mice, potentially contributing to the onset of learning and memory impairment, anxiety, and motor incoordination observed during the disease. The findings propose ligands targeting C1q and C3-mediated releasing activity as potential therapeutic ways for treating synaptopathy in multiple sclerosis patients, offering a novel perspective on complement's involvement in CNS pathophysiology.

Keywords: Complement; glutamate release; EAE mice

Sensorimotor restriction during development induces mitochondrial alteration within muscle and brain structures

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Physical inactivity and sedentary lifestyle during childhood can lead to musculoskeletal and motor deficits, as well as cognitive and behavioral disorders. Most children with developmental coordination disorders or autism spectrum disorders have sensorimotor impairments, reduced physical activity, altered interactions with their environment and atypical motor development. As ATP produced by mitochondria is essential for muscle contraction, neurotransmission, and brain plasticity processes, emerging hypotheses suggest that functional alterations may be related to energy metabolism. Understanding the mechanisms behind these neurodevelopmental deficits is a prerequisite for the development of remediation strategies, which are crucial for promoting self-sufficiency and independence, improving future quality of life and reducing the risk of neurodegenerative diseases in ageing. In order to better understand the emergence of neuromuscular or cognitive disorders during childhood, a model of early sensorimotor restriction has been developed in rats. The model consists in casting pups hindlimbs from birth until postnatal day 28 (P28). These animals exhibit a prominent motor phenotype that includes muscle weakness, locomotor disturbances and altered cognitive and executive functions. Our aim was to determine whether early sensorimotor restriction alters mitochondrial metabolism in rat muscles and brain structures. Enzyme activities of citrate synthase and respiratory chain complexes I, II and IV were measured using a spectrophotometric technique in two hind limb muscles (soleus and extensor digitorum longus) and several brain structures (prefrontal cortex, sensorimotor cortex, striatum, cerebellum, hippocampus) of male and female rats divided into control and in rats early sensorimotor restriction groups, at two developmental stages (P15 and P28). Our results show that early sensorimotor restriction alters mitochondrial metabolism in skeletal muscles, particularly in the soleus, a muscle involved in postural support, but also in different brain structures, with a more pronounced effect in the striatum, involved in movement control, and the hippocampus, involved in learning and memory processes. In addition, a gender effect was observed in several structures. Changes in citrate synthase activity may reflect a change in mitochondrial quantity, and changes in complexes activity indicate that the ATP-producing respiratory chain is less efficient in the affected tissues. In conclusion, the effect of early sensorimotor restriction on mitochondrial metabolism is structure, age, and sex-dependent.

Keywords: Mitochondria, brain, hypoactivity

Multiple Exposome-Wide Association Studies of Psychiatric Disorders in the UK Biobank

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Background Investigating non-genetic factors in mental health has traditionally followed a hypothesisdriven approach targeting selected exposures. This study uses the UK Biobank data to perform multiple Exposome-Wide Association Studies (ExWAS), with the aim to systematically explore the complex network of nongenetic factors associated with psychiatric disorders. MethodsIn this study, we analyzed data from the Mental Health Survey of the UK Biobank (UKB project number: 55392). We focused on self-reported outcomes of various psychiatric conditions, including psychotic disorders, anxiety disorders, bipolar manic episode, depressive disorders, eating disorders, personality disorders, and neurodevelopmental disorders. Following the quality control, 315 variables were included as predictors in the analyses. First, an ExWAS of each psychiatric disorder was conducted in two equally split discovery and replication datasets (Bonferroni corrected $P < 1.59 \times 10\text{-}4$). Subsequently, variables associated with each psychiatric disorder were tested by applying a multivariable logistic regression model per outcome. Results The study population comprised 155,247 participants (57% females; mean age 55.94 years). In the discovery dataset, significant associations were identified across psychiatric disorders: 72 variables (23%) for psychosis, 61 variables (19%) for mania, 186 (59%) for depression, 170 variables (54%) for anxiety, 77 variables (24%) for personality disorders, and 45 variables (14%) for neurodevelopmental disorders. The replication dataset confirmed these associations with high replication rates: 78% for psychosis, 92% for anxiety, 74% for bipolar mania, 94% for depression, 81% for eating disorders, 85% for personality disorders, and 67% for neurodevelopmental disorders. The subsequent multivariable models per outcome demonstrated significant independent associations for 26 variables in psychosis, 65 in anxiety, 21 in bipolar mania, 80 in depression, 35 in eating disorders, 23 in personality disorders, and 16 in neurodevelopmental disorders. Most remarkably, each of the psychiatric disorder categories were associated with both childhood and adulthood traumatic experiences, as well as sleep issues. Cannabis use was also significantly associated with psychosis, bipolar mania, anxiety, and depression but not with personality disorders, eating disorders, and neurodevelopmental disorders. Uniquely, eating disorders showed specific associations with dietary patterns, such as reduced meat consumption, and gastrointestinal issues. For the neurodevelopmental disorders, a particular association observed was increased time spent on the computer. Discussion Our systematic investigation revealed unique and shared associations of mental health outcomes with both well-studied and unexplored variables. Our findings particularly highlight the importance of traumatic experiences for all mental health disorder categories. Our study paves the way for a systematically constructed map of nongenetic factors associated with psychopathology dimensions to guide the future work.

Keywords: Exposome, Childhood Trauma, Environment

Exploring the Impact of Family Experiences and Psychological Indexes on Behaviour towards Antibiotics: A Self-Report Study on an Italian Sample

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Antibiotic resistance represents a worldwide health concern. The World Health Organization (WHO) has estimated around 10 million deaths by 2050 caused by this phenomenon. The main cause for the development of resistance is the prolonged and inappropriate use of antibiotics, which can also affect the individual psychophysiological balance and several biological indices. Given the large number of factors involved in medicine adherence, the purpose of the current study was to investigate beliefs, attitudes, and behaviours of the general population toward antibiotics, through an online investigation, with the aim to identify dispositional factors and personal experiences which influence antibiotics misuse. A sample of 100 responders (74 females, mean age 33.37) completed an online battery of validated scales administered to assess several psychological indexes (Big Five Questionnaire, BFQ-R; State-Trait Anxiety Inventory, STAI; Perceived Stress Scale, PSS; Psychosomatic Problem Scale, PPS), together with a revised form of a WHO survey investigating attitude, knowledge and practice towards antibiotics, and a series of ad hoc questions to investigate disease behaviour and individual and family-related past experiences with antibiotics. Significant correlations between intake behaviour, awareness and individual attitude emerged: having had a family of origin which has demonised antibiotics negatively correlates with the quantity of antibiotics taken in the last year and the personal attitude toward antibiotics, while a family of origin which has used antibiotics improperly positively correlates with the amount of antibiotics taken, and negatively both with the knowledge of good practices of intake and the awareness about antibiotics. Moreover, a good personal past compliance with antibiotic prescription is positively correlated with a family which has used antibiotics correctly. Also, significant correlations emerged between psychological facets and antibiotic behaviour: the personality traits of conscientiousness, agreeableness and openness are positively correlated to antibiotics awareness, on the contrary of indexes of psychological balance (i.e., anxiety, perceived stress and psychosomatization), which correlate negatively to awareness. Moreover, the moderator role of anxiety in the relationship between a negative family approach towards antibiotics and antibiotics intake, and the mediator role of individual awareness in the relationship between a familiar positive approach to antibiotics and individual past compliance to doctor's prescription emerged. Subsequently, regression analysis revealed the role of anxiety, stress and psychosomatization as predictors of individual antibiotics awareness, suggesting that people with a lower psychological well-being may consume antibiotics in a less responsible way. In summary, the present study shed the light on how family experiences and individual psychological factors may play crucial roles in shaping behaviours and attitudes towards antibiotics. These findings can be utilized to formulate patient-centred strategies for therapeutic communication and education.

Keywords: individual factors; antibiotic abuse; antibiotic resistance

2.1. Clinical Neurodegeneration

The Neural Basis of Thermoception

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In October 2019, a patient suffering from frontotemporal dementia (FTD) was admitted to the emergency unit of a hospital in Santander, Spain, for second-degree burns on 30% of the body surface due to scalding in the shower. He was found under the shower by the staff of the nursing home where he was living, fully conscient and standing still under extremely hot water. The treating emergency physician reported that, despite the severity of the injuries, the patient did not complain of pain. Due to the extent of his injuries, the patient passed away1 . This case illustrates how thermoception is crucial for survival, and deficits in thermoception, such as the ones observed in patients with FTD2 or with congenital insensitivity to pain3 can lead to dramatic consequences. Thermoception is a well-defined concept that falls under the umbrella term 'interoception', referring to sensations related to the internal state of the body4 . In addition to temperature, interoception also covers other modalities like pain and has long been recognized a as a key factor in socio-emotional processing5,6 . Interestingly, the clinical phenotype of bvFTD is further characterized by deficits in social cognition, which refers to how social cues are perceived and interpreted7 . The clinical phenotype of bvFTD thus includes deficits in thermoception and socio-emotional processing, supporting a link between both. Furthermore, thermoceptive deficits in FTD have been linked to structural integrity of the insula2 , particularly in the phenotypic variant of the patient reported above, i.e. behavioral variant (bvFTD). Interestingly, the insula is also a key region of degeneration in FTD. There is thus an evident link at the behavioral level between thermoception and the clinical phenotype of bvFTD and at the neural level between the brain regions involved in thermoception and the ones targeted by the neurodegenerative processes of FTD, i.e. the insular cortex2 . The general objective of the project is to provide a comprehensive account of the role of the insula in thermoception and its link with socio-emotional processing by using intracerebral electroencephalography (iEEG) in epileptic patients with insula-implanted electrodes in combination with fMRI in both epileptic and FTD patient cohorts. The convergence of center-specific techniques will ensure optimal temporal and spatial resolution, encompassing neural responses throughout the entire brain. By linking conceptual and methodological levels, this research aims to elucidate the insula's role in thermoception, offering innovative insights into the neural mechanisms governing thermoception, the interplay of thermoception and interoception in socio-emotional processing, and the pathophysiology of FTD. This interdisciplinary approach will provide a comprehensive understanding of these intricate processes, shedding light on the neural dynamics underlying cognitive changes in frontotemporal dementia.

Keywords: Thermoception, Social Cognition, Frontotemporal dementia

Assessing Reading Challenges in Medical and General Texts through Eye-Tracking

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Reading is a crucial component of cognitive processes, serving as a key conduit for individuals to acquire information (K. & Ismail, 2011; Wylie et al., 2018). However, access to and comprehension of information can pose challenges, influenced by factors such as education level, language proficiency, and health conditions (Pandey et al., 2021; Khoong et al., 2019), including neurodegenerative disorders, where reading and comprehension are often significantly impeded (Novakova, 2023; Aarsland et al., 2021). In our research, we propose to investigate the facility of reading and understanding medical and general-language texts. We will employ eye-tracking methodologies, a technique that enables the tracking of gaze patterns and the recording of eye movements during reading. Eyetracking provides objective indicators of reading behavior, such as the duration of saccades, the size of fixations, pupil dilation, and the frequency of regressions (Ekstrand et al., 2021). This study will focus on analyzing variations in reading patterns based on the complexity and technicity of texts. Specifically, we aim to understand how the technicality of language impacts reading efficiency in different populations. Our hypothesis posits that the technical nature of medical texts results in more challenging reading experiences, as evidenced by longer fixation durations, more frequent regressions, and greater pupil dilation. In the image below, we see an example of annotated texts from both medical (Figure 1) and general subjects (Figure 2). The annotation highlights the duration of fixation during reading, indicated by a color gradient ranging from bright green to dark red. Bright green marks shorter fixation durations, while darker shades of red indicate longer durations. As observed, the medical text exhibits longer fixation durations, signified by darker colors, especially at sections that are presumably more complex or challenging for the reader. Figure 1: Medical Text Figure 2: General vocabulary text We have recruited a participant group comprising over 60 healthy individuals. The participants read, using an eye-tracking camera, clinical cases, which represent typical discharge summaries encountered during clinical stays and containing vital health information; as well as texts on general topics from magazines. We also simplified all the texts with a simplification 1 guide to check their actuality and accuracy (Brouwers et al., 2012). The reading experience of clinical cases and general-language texts will be compared. This comparison will focus on three indicators of reading fluency: duration of saccades, size of fixations, and regressions. Additionally, we will examine pupil size as a potential indicator of stress and cognitive load (Yamanaka & Kawakami, 2009). Our research aims to demonstrate whether the technicity of texts significantly impacts their reading and whether stress conditions also influence this process. We suggest that clinical documents, such as clinical cases, should be adapted to the reading and understanding capacity of common people. This assumption is even more crucial for individuals with neurodegenerative disorders. By making medical texts more accessible, we could contribute to better patient education and self-management. Patients with neurodegenerative diseases often face cognitive challenges that can make understanding complex medical information difficult. Simplifying medical texts could significantly aid in patient education and comprehension, thereby empowering patients in their healthcare navigation.

Keywords: medical text simplification, eye-tracking reading analysis, text complexity and comprehension

Exploring ocular glymphatics: The association between intraocular pressure, tear total-tau and MRI-visible perivascular spaces

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Introduction Impaired cerebral waste clearance is a key feature of aging and various neurodegenerative conditions. In Alzheimer's disease accumulation of the waste product total-tau is observed, a protein associated with neuronal damage. Cerebral waste removal operates through a network of perivascular spaces (PVS), where enlarged PVS are an indicator of impaired waste clearance. Recent research suggests the existence of a similar system in the eyes, the ocular glymphatic system. This system is theorized to be driven by the intraocular pressure (IOP), draining waste products from the eye along the optic nerve. This study aimed to gain more insights into the eye-brain connection by investigating the relation between MRI-visible PVS, tear total-tau, and IOP.

Methods MRI acquisition: Thirty cognitively healthy elderly subjects underwent 7T MRI (Siemens Healthineers, Erlangen, Germany), including anatomical whole-brain T2-weighted and T1-weighted scans. Ocular measures: Tear fluid was collected using Schirmer's strips and analyzed for total-tau using ELISA. IOP was measured in the majority of subjects (N = 23) using non-contact tonometry. Perivascular space scoring: PVS were visually rated on the T2-weighted images in the basal ganglia (BG) and centrum semiovale (CSO), two regions known for PVS occurrence. Each hemisphere was scored separately using a visual rating scale: 0 (40). Two independent raters blinded to clinical data performed consensus scoring. Statistics: Partial Spearman's correlation coefficients were computed between the PVS scores and both tear total-tau and IOP, while adjusting for age, sex, tear-wetting length, and their respective hemispheric WM or BG volumes.

Results A higher level of tear total-tau was found to be moderately correlated to a higher CSO PVS score in both the left and right hemisphere (Rs= 0.538, p = 0.004; Rs = 0.546, p = 0.004, respectively). A lower right IOP was moderately correlated with higher right hemispheric CSO PVS scores (Rs = -0.454, p = 0.044), and a similar moderate correlation was found between the left IOP and left hemispheric CSO PVS scores (Rs = -0.474, p = 0.035). No significant associations were found with BG PVS scores. Discussion Higher levels of tear total-tau were related to more PVS in the CSO in both hemispheres. Therefore, elevated tear total-tau may indicate compromised ocular waste clearance, which in turn could signify impaired cerebral waste clearance. This relationship was specific to the CSO, where PVS enlargement is associated with pathological protein deposition (e.g., amyloid angiopathy), as opposed to the BG, where PVS are likely more influenced by vascular changes (e.g., arterial stiffness). Additionally, lower IOP was associated with more PVS in the ipsilateral CSO, aligning with the idea of the presence of an ocular glymphatic system in humans. Lower IOP may reduce the flow of waste-containing fluids through the optic nerve towards the cerebral waste clearance system. While alternative indirect pathophysiological explanations for the IOP-PVS connection should be considered, our exploratory results suggest that a reduction in the pressure driving ocular waste clearance relates to impaired cerebral waste clearance, bridging the gap between these two systems.

Keywords: Brain, eyes, neurodegeneration

Seas of Clarity: the role of brown edible European seaweeds in enhancing cognitive function in progressive MS

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), ultimately leading to neurodegeneration. MS affects over 2.9 million people worldwide of which one million patients are in the progressive phase of the disease (pMS), featured by impaired remyelination and neurodegeneration. None of the currently available approved therapies can repair or regenerate CNS damage in pMS. Since cholesterol is the main component of myelin, alterations in cholesterol metabolism can drive myelin synthesis, facilitate remyelination, and hence protect neurons from degeneration. Noteworthy, the nuclear liver X receptors (LXR) are key players in the regulation of cholesterol and lipid turnover by regulating genes involved in cholesterol uptake, efflux, and transport. In addition, LXRs are implicated in modulating (neuro)inflammation, inducing a positive impact on the environment for remyelination. Recent research showed that the Asian seaweed Sargassum fusiforme, which contains LXR-activating (oxy)phytosterols, is able to prevent cognitive decline and disease progression in a neurodegenerative mouse model without inducing adverse side effects associated with synthetic LXR-agonists. Furthermore, European brown seaweeds were recently reported to have comparable LXR-activating capacities, which makes them an interesting therapeutic intervention for neurodegenerative diseases, such as MS. Here, we will investigate the potential regenerative and neuroprotective capacity of different brown European seaweed species, hypothesizing that the unique bioactive compounds present in brown seaweed may exert neuroprotective effects, ultimately mitigating cognitive decline in pMS patients. In particular, the effect of different seaweed extracts on myelination and remyelination will be assessed in vitro (on iPSC-derived OPCs and neurons), ex vivo (on lysolecithin-treated brain slices), and in vivo (in a cuprizone mouse model). Finally, we will demonstrate proof-of-concept of the most potent seaweed extract in pMS patients. Currently, preliminary data are collected, which will be reported at the conference.

Keywords: Multiple sclerosis; seaweed; cholesterol metabolism.

2.2. Gut-brain axis and Neuroinflammation

Phagocyte-specific TRPV4 deficiency in spinal cord injury repair

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Spinal cord injury (SCI) is a severe life-altering neurological condition where a cascade of inflammatory and pathological processes leads to severe tissue damage and irreversible loss of function. Acutely after injury, phagocytes (microglia and macrophages) have a pivotal role as the primary phagocytic cells to clear the area and protect the glial scar. They are crucial to contain the lesion, but excessive proliferation and their pro-inflammatory character outweigh their beneficial effects, leading to suboptimal recovery after SCI. Interestingly, the lack of the mechanosensory channel TRPV4 (transient receptor potential vanilloid 4) is associated with reduced microgliosis and inflammation at the lesion site, improving functional recovery. TRPV4 is a Ca2+-permeable channel implicated in several microglial functions, such as morphology, motility, proliferation and phagocytosis. Whereas endogenous TRPV4 agonists (i.e. mechanical stress and arachidonic acid metabolites) are found at the lesion site, the individual contribution of microglial TRPV4 in SCI recovery remains unknown. We hypothesize that the increased TRPV4 channel activity at the lesion site leads to excessive proliferation, migration and activation of phagocytes. To disentangle the individual contribution of TRPV4 in phagocytes, we created a phagocyte-specific Trpv4 conditional knockout model and phagocyte-specific TRPV4-deficient bone marrow chimera using a contusive mouse model of SCI. Here we show that a deficiency of TRPV4 in phagocytes does not improve functional recovery after SCI. In addition, microgliosis and scar formation at the lesion site are not reduced. In a full TRPV4-depleted model, we found no improved outcome after SCI either. Altogether, we conclude that the individual contribution of TRPV4 in phagocytes is insufficient to enhance SCI repair.

Keywords: Spinal cord injury, phagocytes, TRPV4

Real-time diagnosis and prognosis of glioblastoma patients with SpiderMass technology

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Glioblastoma is a highly heterogeneous and infiltrative form of brain cancer associated to a poor outcome with a limited efficiency of therapies. Glioblastoma exhibits a median overall survival of 15 month and is classified as a high-grade malignant glioma (grade IV). The extend of the surgery is known to be related to the patient survival. Reaching an accurate diagnostic and prognosis is therefore paramount in the management of glioblastoma. SpiderMass is a technology based on ambient ionization mass spectrometry developed by the laboratory PRISM as a diagnosis or prognosis tool in vivo directly in the operating room. Here, we have studied the performances of the SpiderMass for the diagnosis and the prognosis of glioblastoma patients. For that, 81 glioblastoma excised tissues were analyzed by the SpiderMass technology. The tissue contained either tumor, benign or necrotic area. A specific molecular fingerprint of each tissue is obtained to build classification model capable to distinguish the three types of tissue. We were able to build a classification model with 92% and 88% accuracy for the both ion modes after cross-validation. More interestingly, 95% of correct assignments was obtained on a blind prediction set composed of 9 tissus with 40 regions of interest not unused to trained the model. Combination of supervised and unsupervised analyses allowed to uncover 41 confident lipid biomarkers. After annotation by MS/MS analysis, benign tissue is characterized by the absence of DG instead of the tumor tissues where PA, PE, PS and ceramides are overexpressed. Absence of PI and PC seems to be specific of necrotic tissues. On top of the validated diagnostic based on SpiderMass, we were also interested in investigating the potential to predict the patient outcome. We looked to the patients with the more extreme survivals i.e., 36 months. By narrowing our model to these cases, we obtained 93 % and 87 % of correct classification rate in negative and positive ion mode after cross-validation. This study represents a comprehensive investigation of the possibility to get an accurate histological classification (diagnosis) and prognosis of glioblastoma using ambient ionization mass spectrometry by SpiderMass combined with an artificial intelligence prediction pipeline. This study explores the possibility to accurately diagnosing and prognosing of GBM using SpiderMass alongside an AI pipeline. Employing supervised and unsupervised ML, confident lipid markers are identified and merit further investigation for their therapeutic potential in GBM.

Keywords: SpiderMass – Diagnosis - Prognosis

Mucosal enteric glia migration is affected by microbiota independently of vagal innervation

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Mucosal enteric glia are a sub-population of enteric glia residing in the intestinal lamina propria. These enteric glial cells characterized by type-III morphology have been implicated in the regulation of intestinal barrier function and maintenance of epithelial stem cells. Previously, it has been shown that germ-free mice present with a reduced density of mucosal enteric glia. After conventionalization and restoration of the intestinal microbiome, the population of mucosal enteric glia is re-established by enteric glia originating from the myenteric plexus. This migratory capacity is preserved in physiological conditions and reflects the ordered colonization of the serosa-mucosa axis by ENS progenitors during development. However, the mechanisms that govern their migration and positioning in the mucosa remain obscure. Because of their glial nature, we hypothesized that enteric glial cells require mucosal innervation for their migration towards the lamina propria and maintenance within intestinal villi. To test this, we analyzed the positioning and density of enteric glia in the small intestine of Sox10-CreERT2;Ai14 reporter mice and using immunofluorescence labeling for Sox10 and S100β in whole mounts obtained from germ-free mice and after their conventionalization. In addition, the number and location of mucosal enteric glia were examined in a surgical model of unilateral sub-diaphragmatic vagotomy, which was employed to eliminate the extrinsic neuronal component of the lamina propria. vGlut-Flp;Phox2B-Cre;Ai65 mice, expressing the fluorescent reporter tdTomato in afferent fibers of the vagus nerve specifically, were used to determine the effectivity of the surgeries and to examine the association between vagal afferents and mucosal enteric glia. In line with earlier reports, we found a reduced density of mucosal enteric glia in the villi of the ileum of germ-free mice, with the remainder of glia located close to the villus base. A similar result was observed in the jejunum, but not in the duodenum. Notably, the absence of microbiota was associated with an increase in villi length in the duodenum and ileum. Unilateral vagotomy did not affect mucosal or myenteric glia density in control animals, nor did it impact the colonization of the lamina propria by enteric glia induced by conventionalization of germ-free animals. Interestingly, conventionalization of germ-free mice resulted in an increased glia density in duodenal lamina propria, but not in the jejunum. A reduction and absence of mucosal vagal afferents demonstrates that the, respectively, unilateral subdiaphragmatic and bilateral vagotomy were successful. We provide new insights into the interaction between microbiota and mucosal enteric glia. Our results indicate that microbiota shape the architecture of the intestinal mucosa. In addition, by performing region-specific vagotomy experiments, we demonstrate that mucosal enteric glia appear not to require vagal innervation for their migration or presence in the lamina propria of the mouse small intestine. Our current experiments focus on the histo-spatial interactions and cellular activity patterns instructing mucosal enteric glia homeostasis.

Keywords: Enteric glia – Enteric nervous system – microbiota – Vagus nerve

Gastrointestinal dysbiosis and functional alterations in a mouse model for psychiatric disorders

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Introduction: Neurodevelopmental disorders are often accompanied by gastrointestinal (GI) comorbidities. However, the precise mechanisms underlying GI symptomatology in these central nervous system-impacting conditions remain elusive. Aberrant expression of Disrupted in Schizophrenia 1 (DISC1), a hub and scaffold protein which plays key role in neural maturation and connectivity, serves as a critical risk factor for several psychiatric conditions. We aim to elucidate whether and how DISC1 perturbation contributes to alterations in gut function, focusing on enteric nervous system integrity and GI homeostasis.

Methods and materials: Adult DISC1 locus impaired (LI) mice were compared with wild type (WT) littermates by using mRNA and protein expression analyses, standard histological evaluations, and in vivo assessments of GI functionality. Stress levels were quantified by ELISA, measuring corticosterone levels. Linear discriminant analysis scoring was employed to characterize intestinal microbiota composition.

Results: First, knock-down of DISC1 mRNA levels in whole gut tissue of DISC1 LI mice was confirmed. Anthropometric measurements revealed decreased body weight, small intestine, and colon length in DISC1 deficient animals. Evaluation of whole gut transit time uncovered that LI mice have faster GI transit ($P=0.0347$). However, the colonic bead propulsion assay did not show differences in distal colonic motility. Notably, LI mice exhibited diminished wet stool weight relative to WT littermates ($P=0.0086$), with no discernible alterations in stool water content or intestinal barrier function. Haematoxylin and eosin staining excluded any histopathological alterations and inflammation as an underlying factor of the altered gut function. Consistently, no differences in glial fibrillary acidic protein levels were observed. Immunofluorescence labelling showed elevated numbers of myenteric neurons in mutant mice relative to WT littermates ($P_{Small\_Intestine}=0.0939$, $P_{Colon}=0.0101$). However, intestinal microbiome analysis revealed enrichment of Bacteroides ($P<0.05$), and reduced levels of Muribaculaceae ($P<0.05$) and Clostridia ($P<0.05$) in LI mice. Corticosterone hormone levels were similar between LI and WT littermates.

Conclusion: Our data indicate that DISC1 disruption results in faster GI transit, a higher density of myenteric neurons and dysbiosis, but does not induce intestinal inflammation, mucosal barrier dysfunction or increase corticosterone levels. Notably, the enriched bacterial taxa within the respective genotypes are phylogenetically affiliated and the microbiota profile identified in DISC1-deficient mice is characteristic observed in human studies examining psychiatric patient cohorts. Currently, our ongoing investigations aim to determine the cellular origin of DISC1 within the gut. In addition, we aim to normalize GI homeostasis in DISC1 LI mice by rescuing microbial dysbiosis through faecal microbiota transplantation interventions.

Keywords: psychiatric disorders, gastrointestinal dysfunction, microbiota-gut-brain axis

Poster session abstracts

Welcome to the Poster Session Abstract of our booklet!

In this section, we showcase a curated collection of research summaries, presenting a diverse array of innovative ideas, methodologies, and findings. Each abstract encapsulates the essence of its respective poster, offering a glimpse into the exciting world of academic exploration that awaits you at our event. Join us in unraveling the intricacies of these thought-provoking studies, where creativity meets intellect, and ideas spark inspiration.

These abstracts belong to the corresponding parallel session from the PhD talk parallel sessions, feel free to dive in and go have a chat with the authors during the Poster sessions. You will not be disappointed.

Spatial multi-omics guided by SVD k-means ++ clustering and statistical estimation of heterogeneity: Rat Brain sections as playground

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Integrating machine learning tumor heterogeneity in the cancer diagnosis process is an essential issue to tackle the complexity of the pathology with precision and efficiency. Specific omics molecules identifications for each heterogeneous tumor subpopulations are especially important in order to characterize them in depth. In this way, distinguishing and detecting one clone from another, whatever the molecules analyzed, is an easy and fast process which can be carried out during the diagnosis. Moreover, protein information from each subpopulations are particularly interesting for drug discovery to tailor optimized therapy to the heterogeneous tumor.

For this purpose, the implementation of the concept was first optimized on rat brain (RB) sections. Lipid, protein and peptide MALDI mass spectrometry imaging (MSI) were performed on 5 cerebellum area rat brain replicates. A first MatLab script was developed to process imaging data, providing unsupervised clustering, independently for each data set. 3 clusters were identified with the same spatial location for each images, whatever the molecule analyzed, corresponding to the Granular Layer (GL), Molecular Layer (ML) and the White Matter (WM) region of the cerebellum RB. The integration of the Silhouette criterion prediction, also allowed to determine the most optimized segmentation number of cluster, in a completely unsupervised manner. This algorithm is very effective in generating optimal segmentation images, even on tissues more complex than the RB cerebellum.

A second Python script provided the lipid, protein and peptide discriminant cluster ions. Microproteomic analysis, and Back side analysis using SpiderMass technology, were performed to identify the protein ions previously listed. MS/MS analysis were also performed with SpiderMass to identify the discriminant lipids. Finally, the multi MSI made it possible to demonstrate the presence of various heterogeneous subpopulations, each presenting a specific localized lipid and protein network. This information will make it possible to produce a computer prediction model, which will make it possible to identify the presence of any cluster on any tissue according their specific molecular fingerprint, and validate it by machine learning.

Finally, this tool combined to a home-made lipid associated protein data base, would be useful to analyze complex tissues. Indeed, the future research effort will be to focus on cancer tissues, as glioblastoma for example. Our goal is to perform MSI on the tumoral tissue, and interrogate the prediction model to automatically generate the cluster identification, as well as the associated lipid and protein pathways. By this new idea, we introduce the concept of dry proteomic based on the identification of their conjugated cluster lipids.

Keywords: Spatially resolved MALDI mass spectrometry imaging, Machine learning, SPIDERmass, Lipidomic and proteomic interaction network.

Development of a synthetic biomarker-based immunotherapy in glioblastoma

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Glioblastoma is the most aggressive and common type of adult brain tumor. Conventional treatment consists of total or partial resection of the tumor, followed by radiotherapy and chemotherapy. Unfortunately, these approaches have little impact on the prognosis of patients. The advent of immunotherapies has led to spectacular advances in the treatment of many cancers. However, in the case of glioblastoma, the results of clinical trials have not been encouraging and this can be explain by two particularity of glioblastoma. First, there are few known tumor antigens specific to glioblastoma, and when used as therapeutic targets, they show low efficacy and severe side effects. Secondly, the glioblastoma microenvironment is highly immunosuppressive and is called "cold". There is a strong expression of immune checkpoint inhibitors and significant secretion of immunosuppressive cytokines. There is also a significant infiltration of TAMs (Tumor Associated Macrophages), mainly derived from bone-marrow-derived-monocytes.

As promising immunotherapies, CAR-T cell therapies have shown spectacular results in liquid tumors. However, results in solid tumors have been less promising. To overcome the problems encountered by CAR-T cells, it has been tested to use macrophages as CAR carriers. It has been shown that CAR macrophages (CAR-M) are able to phagocyte cancer cells expressing the target antigen, thereby enabling tumor elimination in vivo. Characterization of CAR-M activity has shown that they secrete pro-inflammatory cytokines and chemokines, convert M2 macrophages to M1, act as antigen-presenting cells and are resistant to immunosuppressive cytokines. Macrophages are therefore a good alternative to T cells in the treatment of solid tumors such as glioblastoma.

However, CAR therapies depend on the identification of a tumor antigen. Ideally, this antigen should be exressed uniformly, prominently and selectively on tumor cells. The choice of target will determine the toxicity of the treatment by inducing an "on-target/off-tumor" effect that will induce the destruction of healthy tissue by the CAR cells. Frequently targeted tumor antigens such as Her2 or EGFR are also expressed at low levels by healthy cells and are, therefore not ideal targets.

The aim of this project is therefore to induce the expression of an exogenous antigen on the surface of cancer cells and to target this antigen with a CAR-M cell. Several exogenous antigens can be used, such as bacterial beta-galactosidase, mCherry protein or an HA tag. To make these proteins membrane-bound and accessible to the immune system, they must be coupled to a membrane protein such as connexin43 or the transmembrane domain of the transferrin receptor. As these proteins are not expressed on the membrane of human cells, targeting these antigens does not cause toxicity in healthy tissues. To target only the tumor, the idea is to use a recombinant adeno-associated vector (AAVr) to transfer the sequence and thus induce expression of the exogenous antigen only in tumor cells. The tumor cells expressing the antigen will be then targeted by CAR-M
In conclusion, by inducing the expression of an exogenous antigen, we will be able to make cellular immunotherapeutic strategies efficient for difficult-to-treat solid tumors such as CNS tumors.

Key words: glioblastoma; chimeric antigen receptor; synthetic antigen

Mechanisms of generation of a new truncated form of the Tau protein in Alzheimer's Disease

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Introduction

Tau protein is a central actor in Alzheimer's disease (AD) pathophysiology. A new pathological truncated form of Tau called AcMet11-Tau was discovered by the laboratory but the mechanisms leading to its production are not elucidated. The hypothesis is that AcMet11-Tau is generated by alternative translation under stress conditions, especially Integrative Stress Response (ISR). Accordingly, our preliminary results show that when a stable Human neuroblastoma cell line containing Tau cDNA is exposed to Sodium Arsenite (SA), the ISR is induced and the AcMet11-Tau is generated. So, the objective of this project is to investigate in different experimental models the impact of ISR on Tau translation and the role of alternative translation mechanisms in AcMet11-Tau generation.

Methods

We use SA treatment to induce ISR in Lund human mesencephalic (LUHMES) cells, that can be differentiated to post-mitotic neurons. To analyze AcMet11-Tau production from human endogenous Tau, we use Western Blot, ELISA, qPCR, and ribopuromycylation methods combined with Protein Ligation Assay (PLA). Also, we are investigating the effects of SA treatment *In vivo* by using transgenic mouse models of Tau pathology. We analyze the effects of SA treatment on the generation of AcMet11-Tau as well as on Tau pathology and its related cognitive deficits.

Results/expected results

Preliminary results from human neuroblastoma cell lines (SY5Y/TR Tau) show that AcMet11-Tau is indeed generated by alternative translation after SA treatment. Our studies in neuronal cells and *In vivo* will establish the role of physiologically relevant neurodegenerative stress inducers in the deregulation of Tau translation.

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Keywords: Tau protein, stress, alternative translation

Interplay of metabolic stress cascade and autophagy in synaptic physiology

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A complicated robust nutrient sensing network has evolved to withstand changes in metabolic availability to maintain plasticity and cognitive function throughout one's life. However, this networks ability to sense and adapt declines with ageand may contribute to the acceleration of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease. While each specific neurodegenerative disease exhibits distinct features and symptoms, emerging evidence suggests that shared or interconnected pathways exists in the early stages of disease onset. These shared characteristics are vast but include dysfunctional autophagy linked to metabolic dysregulation resulting in vulnerability of synaptic function and loss of axonal integrity. Sensing and maintaining appropriate local metabolic conditions across compartments is crucial for neuronal health and may contribute to variable compartment vulnerability contributing to the onset the aging and the development of neurodegenerative diseases.

Our aim is to investigate the molecular mechanisms underlying neuronal physiology and explore the varied response of neuronal compartments (i.e. soma *versus* synapses) to metabolic stress under autophagy deficiency using wild type and ATG5 KO cortical and hippocampal neurons. To address our objectives, we utilized techniques such as immunohistochemistry, live cell imaging, whole proteomic and phosphoproteomic analysis and EM. Our results indicate that after a short period of selective nutrient deprivation (complete withdrawal of amino acids (AA)) neuronal survival is significantly compromised. AA withdrawal causes neuronal cell death, defective nutrient sensing via mTOR signaling, accumulation of abnormal mitochondria at synapses and reduced ATP production. Significantly, this AA withdrawal induced phenotype can be rescued by the additional removal of serum.

Keywords: Synaptic Physiology, Metabolism, Autophagy

Investigating Targeted Gene-Expression Alterations Effect in In Vitro Scalable Co-cultures

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The complex molecular mechanisms regulating the Excitatory/Inhibitory (E/I) balance in the brain, crucial for neurodevelopment and implicated in diverse disorders, are the core focus of this study. We specifically aim to elucidate gene-dependent modulations of the E/I balance, particularly exploring implications for neurodevelopmental disorders including ADHD, specific epilepsy types, and intellectual disability. Our research centers on the St3GAL3 gene, identified as a prominent candidate in GWAS analysis.

Utilizing CRISPR/Cas9, we executed targeted knockout of St3GAL3 in healthy induced Pluripotent Stem Cells (iPSCs). This approach allows for parallel differentiation of iPSCs into diverse neuronal subtypes. The isogenic control line provides a crucial reference for comparative analysis.

To ensure robustness, two complementary approaches were employed for iPSC differentiation. First, a small-molecule-based method induced a heterogeneous population of cortical neurons and astrocytes. Second, lentiviral induction facilitated the creation of pure monocultures or co-cultures of Glutamatergic and GABAergic neurons. Morphological, molecular, and functional assessments were conducted to scrutinize the role of St3GAL3 in the E/I balance.

Functional assessments were particularly comprehensive, utilizing Multi-Electrode Arrays to measure extracellular electrophysiological activity. This comprehensive investigation into St3GAL3's influence on the E/I balance contributes valuable insights to the understanding of neurodevelopmental disorders and potential therapeutic avenues.

Keywords: iPSCs, CRISPR\Cas9, Electrophysiology

Contribution of ventral spinal interneurons to the etiopathogenesis of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is an adult-onset devastating neurodegenerative disease primarily characterized by the death of upper and of lower motor neurons (MN), leading to progressive muscular paralysis. First considered as a MN specific disease, animal models and *in vitro* or cell transplant studies demonstrated that other cell types surrounding MN could contribute or even be causative of the disease through non-cell autonomous mechanisms.

In the spinal cord, ventral interneurons (IN) regulate MN activity via excitatory or inhibitory synapses, allowing an appropriate motor response. Recently, growing interest in spinal IN involvement in the context of ALS emerged, yet their contribution to the pathology remains largely unsolved. We hypothesize that ventral spinal IN are either causal agents or modulators of alterations leading to ALS. In the frame of my PhD project, I will focus on V0 IN, a specific IN population composed of different subtypes (excitatory, inhibitory or cholinergic neurons) sending synaptic projections onto MN or other IN.

The goal of my thesis is to determine the contribution of V0 INs to the etiopathogenesis of ALS, using on the one hand an existing SOD1 transgenic mouse model and on the other hand an innovative TDP-43 mouse model I plan to generate. This study should provide critical information about the molecular and cellular mechanisms of ALS that are necessary for an early diagnosis and for the identification of novel treatment options, as well as a new mouse model to study the pathology.

Keywords: ALS, Spinal cord, Interneurons

Genome Integrity and Neurological Disease

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Neurological complications directly impact the lives of hundreds of millions of people worldwide. While the precise molecular mechanisms that underlie neuronal cell loss remain under debate, evidence indicates that the accumulation of genomic DNA damage and consequent cellular responses can promote apoptosis and neurodegenerative disease. This idea is supported by the fact that individuals who harbour pathogenic mutations in DNA damage response genes experience profound neuropathological manifestations. The central nervous system (CNS) is protected from most exogenous threats to neuronal DNA by the vertebral column, the skull, and the blood-brain barrier. Therefore, the CNS is mainly challenged by endogenous stressors. Since the brain consumes a lot of oxygen for ATP production, reactive oxygen species (ROS) are abundantly produced as a byproduct of the electron transport chain. I will show that ROS can induce DNA damage in the forms of interstrand crosslinks, strand breaks, abasic sites, and base modifications. The accumulation of DNA damage in neuronal cells either drives the loss of genome integrity or interferes with gene regulatory processes or transcriptional events, thereby promoting activation of cell death responses and consequent cell loss. Depending on the proliferative nature of cells, a preference for certain DNA repair pathways exists. I will elaborate on how dividing cells regularly use systems such as mismatch repair (MMR), ribonucleotide excision repair (RER), and homologous recombination (HR), whereas non-dividing cells favour non-homologous end-joining (NHEJ), single strand break repair (SSBR), or nucleotide excision repair (NER). Not surprisingly, given that neurons are non-dividing in nature, defects in DNA repair pathways such as SSBR and NER, which resolve obstructive DNA lesions largely independently of replicative status, have been linked to neurodegenerative outcomes. It is important to recognize that the nervous system is composed of an integrated network of neuronal and glial cell types. Determining the consequences of persistent DNA damage and the comparative role of the different DNA repair mechanisms in the distinct cell populations represents a critical step in defining the factors that influence health of the nervous system as a whole. A broader understanding of DNA damage and repair within the cellular network will not only shed new light on the aetiology of neurodegenerative diseases but can pave the way for new therapeutic strategies. My recent effort has worked to uncover the role of DNA damage and DNA repair following spinal cord injury (SCI). It seems that DNA damage, as measured by the marker gamma-H2AX, is present around the spinal cord lesion from one hour post-injury until three days post-injury. This damage then spreads outward over time from the lesion site to more distal regions of the spinal cord. Cell type-specific analysis points out that 30-40% of all neurons experience DNA damage at an early timeframe. These data collectively indicate that DNA damage occurs very rapidly following SCI and possibly contributes to the secondary neuronal loss seen at late stages in the pathophysiology of SCI. As will be highlighted, the results give clear indications for the therapeutic window of DNA repair-related interventions for SCI patients.

Keywords: oxidative DNA damage; DNA repair; neurodegeneration

Exploring the Impact of Influenza virus and Gammaherpesvirus Infections on Exacerbating Experimental Autoimmune Encephalitis

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The Epstein-Barr Virus (EBV) is one of the most prevalent virus worldwide with over 90% of the global population being infected and is the cause of mononucleosis. While a long-term infection remains asymptomatic in most individuals, a recent 20-year study on 10 million US army personnel revealed a 32-fold increased risk of developing multiple sclerosis in EBV-seroconverted individuals. MS is a complex autoimmune disease affecting 2.8 million people worldwide, primarily young adult women. Its intricate aetiology involves genetic predispositions and environmental factors, with EBV showing the strongest MS association. The focus of this study was to confirm EBV's unique role in MS pathogenesis using a related murine virus, known as Murid Herpesvirus 4 (MuHV-4), in mice treated for developing Experimental Autoimmune Encephalitis (EAE), a relevant model for MS. While previous experiments showed that MuHV-4 worsens the EAE disease course, we aimed here to establish MuHV-4's unique contribution by comparing it with influenza virus infection in mice. EAE was induced 30 days post-infection, and disease progression, along with immune cell activation, was monitored in various tissues. Our experiment confirmed MuHV-4's distinct impact on EAE development, emphasizing the critical role of EBV in increasing MS risk. More generally, our project delves into understanding EBV's role in MS development and its influence on brain myeloid cells.

Keywords: Multiple Sclerosis; Murid Herpesvirus-4; Influenza Virus

Unravelling Downstream PDE4D-cAMP Signalling in the Context of Neuro- and Myelin Regeneration in Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune disease resulting from immune attacks on myelin within the central nervous system (CNS). The myelin sheath, produced by oligodendrocytes, insulates neuronal axons and is essential for neuronal protection and efficient signal transduction. This underscores the significance of an interconnected relationship known as the oligodendrocyte-neuron unit. In MS, myelin-producing oligodendrocytes are destroyed, leading to demyelinated CNS lesions and axonal damage. While partial remyelination is possible in early stages through oligodendrocyte precursor cell (OPC) differentiation, disease progression and recurrent harm to the oligodendrocyte-neuron unit lead to chronic demyelination and neurodegeneration. Current anti-inflammatory drugs fail to halt disease progression or reverse neurodegeneration, necessitating targeted neuro-and myelin regenerative MS therapies.

The second messenger 3'-5'-cyclic adenosine monophosphate (cAMP) plays a pivotal role in inflammation, neuroplasticity, and cellular differentiation. cAMP levels are regulated intracellularly by phosphodiesterases (PDEs), a superfamily of enzymes consisting of 11 families (PDE1-11). Recent findings demonstrate that inhibiting the breakdown of cAMP via Gebr32a, a PDE4D-gene specific inhibitor, promotes *in vitro* neurite outgrowth, OPC differentiation, and remyelination. However, the precise cAMP-triggered signalling pathway following PDE4D inhibition remains unknown. While PDE4D inhibition holds promise for MS treatment, preclinical studies show severe adverse effects. Therefore, understanding key players in neuro-and myelin regenerative pathways is crucial to find new targets.

This project seeks to identify signalling pathways activated by elevated cAMP levels through PDE4D inhibition using Gebr32a and RIC01 in neurons and oligodendrocytes. To this end, human induced pluripotent stem cells (hIPSCs) will be employed. First, we aim to investigate which primary downstream effector of cAMP, namely protein kinase A (PKA) or exchange protein activated by cAMP (EPAC), mediates enhanced OPC differentiation and neurite outgrowth. Secondly, we will explore phosphoproteome and transcriptome dynamics following PDE4D inhibition by means of mass spectrometry and RNA sequencing, respectively. Third, CRISPR/dCas9 will be used to manipulate target gene expression through transcriptional activation or repression, assessing the functional significance of identified pathways. This project will uncover key phosphoproteins and genes driving neuro- and myelin regeneration upon PDE4D inhibition, discovering novel, effective, and clinically safe therapeutic targets to restore the oligodendrocyte-neuron unit in MS patients.

Keywords: multiple sclerosis, cAMP signalling, PDE4D

Characterisation of Central Chemoreception and Cardioregulation in Sudden Unexpected Death in Epilepsy

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Central apnea emerges to be the key contributor for Sudden unexpected death in epilepsy (SUDEP). Particularly dysregulation of central ventilatory chemoreception (CVC), the system detecting changes in PCO_2 levels, is postulated to play a role in SUDEP, accompanied by autonomic anomalies such as cardiac dysregulation. Currently, vagus nerve stimulation (VNS) is used as an adjunctive treatment for SUDEP and is also shown to activate chemosensitive and cardioregulatory pathways. This research project aims to fill in the gaps in the molecular understanding of SUDEP pathophysiology, particularly focusing on central chemoreceptive and cardioregulatory centers.

The project aims (1) to investigate the progression of CVC and heart rate in relation to epilepsy duration hence severity in kainic acid (KA)-induced rats; (2) to explore the immunolabelling of neuromodulators in central chemoreceptive and cardioregulatory regions and their response to CO2 in epileptic rats and healthy controls and (3) to compare the vagus nerve electroneurogram (VENG) signal in high CO_2 condition in epileptic and healthy animals.

The study utilizes an established rat model of epilepsy induced by intrahippocampal KA injection. Ventilatory responses to CO2 and heart rate variations are measured via photoplethysmography alongside with EEG to assess effects of epilepsy progression. Immunolabelling techniques are employed to identify neuromodulator expression patterns and activation responses in cardioregulatory regions.

The results from this study are anticipated to provide insights into the relationship between epilepsy duration and alterations in CVC and cardioregulation. This project will advance the understanding of SUDEP mechanisms and open pharmacological avenues for therapeutic interventions. Additionally, assessing VENG in high CO_2 will contribute to the development of vagus nerve based SUDEP detections to improve the efficacy of VNS.

Keywords: central chemoreception, epilepsy, SUDEP

Targeting of tau protein using anti-PHF6 VHH : intrabody or minibody approaches

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Tauopathies are a group of neurodegenerative diseases characterized by an intracellular accumulation of tau protein aggregates. In some tauopathies, the spatio-temporal progression of the pathology and the presence of the tau protein in biological fluids has led to the hypothesis of a “prion-like” propagation. However, in other tauopathies, the progression is more debatable and the tau protein is difficult to detect in the fluids suggesting that therapeutical approaches should be different. To limit the progression of tau pathology, classical immunotherapy is increasingly used in clinical trials and the development of news specific strategies are now ongoing.

VHHs (variable heavy chain of antibodies from camelids) and their possible modification in the form of a minibody (containing the Fc part) can target the tau protein respectively inside the cells or in the interstitial liquid that surrounds the cells in the brain. In this scientific context, the targeting of the PHF6 epitope of the tau protein, involved in the formation of fibrillar aggregates, was selected to test these two approaches (intrabody/minibody) in a tau transgenic mouse model.

An intrahippocampal injection of viral vectors encoding anti-tau VHH was done in a tau seeding mouse model. One month later, mice were sacrificed and immunostaining revealed different level of reduction of tau pathology in hippocampus depending of the VHH construct (mini or intrabody).

We then tested these VHHs for a long-term study in tau transgenic mouse after viral vectors delivery in hippocampus. Phenotyping, cerebral imagery, behavorial studies and tau pathology post-mortem analyses point out the efficiency and the safety of this treatment.

The difference observed between the different approaches of anti-tau VHH immunotherapies raises questions about the stability of VHH and the choice of subcellular compartment targeting.

Keywords: tauopathies, intrabody, minibody

Role of extracellular vesicles in the brain metastasis tropism of triple negative breast cancer

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Triple-negative breast cancer (TNBC) is one of the breast cancer subtypes with no expression of genes encoding to hormone receptors (progesterone & estrogen) and EGF type 2 receptor. It represents approximately 20% and is considered as the most aggressive and metastatic subtype. TNBC exhibits metastases in preferential target organs, including bones, lungs, liver, and brain. However, a chemoresistance process appears during chemotherapy, leading to an alarming increase in brain tropism. The prevalence of brain metastasis for patients is 70% five years after diagnosis. Moreover, brain metastases are difficult to target due to the inadequate and limited permeability of the blood-brain barrier for therapeutics which accelerates the lethality. As a result, brain tropism remains among the most aggressive step and represents a major cause of death. The spread of tumor cells is a non-random process. In order to colonize a specific site, primary tumor cells require the preparation of a pre-metastatic niche (PMN) before their migration. The underlying molecular mechanisms involved in the conditioning of this PMN, as well as the messengers delivered in return to recruit tumor cells, are not well described. Recent studies suggest the importance of extracellular vesicles (EVs) in this molecular communication. EVs are represented by two main populations, ranging from 50 to 150 nm for exosomes and 50 to 1000 nm for microvesicles. EVs are important messengers and carriers of biological signals at distance by providing effectors or regulators as proteins, lipids and nucleic acids.

This project aims to understand the molecular mechanisms involving EVs derived from primary TNBC cells, in the targeting of potential PMN in brain. Of note, proteins on surface of EVs play an important role in docking with target cells at a specific secondary site during the PMN. EVs from a parental cell line of human TNBC (MDA-MB-231-TGL) were compared to those of its derivative brain metastatic cell line (MDA-BrM2-831), established after the injection of the parental cell line in nude mice and the collection of metastatic clones in brain. The comparison of distinct EV populations are based on the characterization of surface and intravesicular proteins and aim to better understand specific changes in EV subtypes to establish PMN in brain. In the context of a possible cerebral tropism dependent on EVs, they were used to challenge astrocytes and microglia and investigate the subsequent protein changes and secretomes.

The first results suggested the production of differential EV surfaceomes. In addition, CCL22, VEGF, CXCL5 and IGFBP2 in astrocytes were upregulated by EVs derived from brain metastatic cell line. In the literature, (i) CCL22 is known to chemoattract tumor cells, (ii) VEGF is found to increase the permeability of BBB and promote brain metastasis, (iii) CXCL5 promotes the recruitment of tumor cells and immune cells and (iv) IGFBP2 is also secreted by astrocytes, as IGF1 cofactor, to finally promote the proliferation of brain metastatic cells in the nerve tissues. The specific inhibition of EV surfaceomes would give a new insight into the PMN physiopathology.

Keywords: Brain metastasis, Extracellular vesicles, Glia

Phosphodiesterase 4B and 4D Inhibition: A Sequential Approach to Unlock Spinal Cord Injury Repair

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Spinal cord injury (SCI) patients suffer from permanent or transient neurological deficits, leading to a drastic reduction in their life quality. Modulating the acute neuroinflammatory response and boosting endogenous repair mechanisms are two key strategies to promote SCI repair. **Cyclic adenosine monophosphate (cAMP)** is a second messenger involved in both inflammatory and regenerative processes. Intracellular cAMP levels are spatiotemporally controlled by its hydrolyzing enzymes phosphodiesterases (PDEs), with PDE4 being the most abundantly expressed PDE in the central nervous system (CNS). Interestingly, cAMP is severely reduced after SCI, but preserving cAMP levels through **PDE4 inhibition was demonstrated to improve SCI outcomes** in rodents by attenuating the inflammatory responses and promoting regeneration. Unfortunately, the clinical translation of PDE4 inhibitors is hampered because of severe side effects. However, inhibition of PDE4 subtypes, PDE4B or PDE4D, circumvents the emetic side-effects observed with pan PDE4 inhibition. Additionally, PDE4B inhibition demonstrated to reduce inflammatory responses, while PDE4D inhibition showed to promote repair mechanisms in other CNS disorders. Hence, subtype-specific PDE4 inhibition provides new opportunities to treat SCI. **We hypothesize that inhibiting PDE4B in the early phase, followed by PDE4D inhibition in a later stage, will attenuate inflammation and promote regeneration, respectively, leading to improved SCI outcomes.**

Our preliminary data already revealed that PDE4D inhibition starting ten days post-injury (dpi) improved SCI outcomes, decreased the demyelinated area, and reduced the lesion size in a hemisection SCI model. However, regeneration is postulated to enhance SCI outcomes further. Therefore, we will **first investigate the optimal therapeutic window (dose and timing) of the PDE4D inhibitor to promote regeneration in a hemisection SCI model.** Functional outcomes will be examined using the Basso Mouse Scale (BMS) score, and axonal dieback of Fluoro-ruby-labeled corticospinal tract fibers will be quantified.

Once the optimal therapeutic window of the PDE4D inhibitor is achieved, we will **investigate the therapeutic potential of sequential PDE4B inhibition, followed by PDE4D inhibition using the contusion SCI mouse model.** The BMS score and the CatWalk XT system will be used to analyze functional outcomes. Additionally, neuronal integrity and myelination will be accessed by measuring somatosensory evoked potentials (SSEP) in the motor cortex upon a peripheral stimulus. Lastly, demyelinated area, lesion size, and neuronal sprouting will be investigated post-mortem, while the immune cell phenotype will be examined with flow cytometry.

Lastly, **the effect of PDE4B and PDE4D inhibition will be elucidated on microglia, oligodendrocytes, and neurons isolated out of the injured murine spinal cord.** Cellular phenotype, e.g., differentiation and inflammatory status, and functional outcomes such as phagocytosis, myelination, and outgrowth will be used to determine the direct effect of subtype-specific PDE4 inhibitors on spinal cord-derived cells. In this way, we aim to get more insights into the therapeutic potential of sequential subtype-specific PDE4 inhibition to treat SCI.

Keywords: Spinal cord injury, cAMP, phosphodiesterase 4, neuroinflammation, regeneration

Development of an in vivo ImmuneScore in the case of Glioblastoma via the SpiderMass technology

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Infiltration of various population of immune cells was found to be associated to the patient outcome in colon cancer and is now recognized in more and more cancers. Nowadays, the ImmuneScore is obtained post-surgery from excised tissues or biopsies by immunohistochemistry (IHC) using antibodies specific of the different immune cell populations.

SpiderMass is a technology based on ambient ionization mass spectrometry developed by the laboratory PRISM as a diagnosis or prognosis tool *in vivo* directly in the operating room. Here, we explored the possibility to create an ImmuneScore based on SpiderMass data that could in the future be exploited *in vivo*. To this end, we analyzed with SpiderMass different population of immune cells namely macrophages (M1-like and M2-like), lymphocytes versus NCH82 glioblastoma cells.

A Python library called LGBM was used to train an immune scoring model for which a correct classification rate of 100% was obtained. Using this model, we were able to find immune cells lipids biomarkers like the ions *m/z* 818.65 (GlcCer d18:1_22:0) and 819.55 (PG 18:1_22:6) specific M1-like and M2-like macrophages respectively.

Most interestingly, from a SpiderMass images, we were able to get the predicted distribution of the immune cells across the tissue. Indeed, a pipeline was dedicated to predict the probability of presence of each cell type based on SpiderMass images of 6 FF glioblastoma tissues. The ratios provide insight into the distribution of the trained cell types across the image, allowing a comprehensive assessment of the cellular landscape in patient with shorter versus longer survival.

Moreover, the lymphocytes were predicted to have a higher abundance in tissue from patients with >36-month survival as well as M1-like macrophages. Conversely, M2-like macrophages is highly expressed in patients with shorter survival. Finally, the ratio of M1-like to M2-like macrophages could serve as a prognosis marker.

Our newly developed immunescoring pipeline was confirmed thanks to a 5-plex MALDI-IHC panel. It corroborates our result thanks to the use of antibodies conjugated with novel photocleavable mass-tags. Of particular interest, the SpiderMass-MSI approach, based on immunescoring, allows the differentiation of various subpopulations within the tumor microenvironment without necessitating techniques reliant on probe utilization. In conclusion, this innovative approach not only offers insights into the presence of immune cells within the TME but also presents the potential for a more rapid prognostication of survival time among GBM patients.

Keywords : Mass spectrometry – Artificial intelligence - ImmuneScore

The effect of PS2 deletion or mutation on the lipidic profile

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Alzheimer's disease (AD) is the most common form of neurodegenerative dementia, accounting for 60% of diagnoses. Presenilins (PSs) 1 and 2 are major players in the progression of amyloid pathology, a characteristic lesion of AD. PSs are the catalytic subunits of γ-secretase, which produces amyloid peptide (Aβ) – responsible for the formation of amyloid plaques – by cleaving the amyloid precursor protein (APP). Mutations in PSs found in inherited forms of AD have been the subject of numerous studies. Some suggest a partial loss-of-function effect of PSs in AD whose pathogenic effect is not exclusively associated with their role in Aβ production. Alterations in mitochondrial function and lipid profile have been shown in PS-deficient cell models, mainly related to PS2 deletion. Lipid metabolism is deeply involved in the biological aging process. Our aim is to better understand the role of cellular lipid metabolism controlled by PSs, and more specifically PS2. Therefore, we are working with PS2 -/- and PS2N141I mice, expressing the N141I mutation. First, based on preliminary results obtained from a microarray analysis that identified an upregulation of Lpl and Smpd3 in PS2 /- MEF (Mouse Embryonic Fibroblasts) cells, we have evaluated their protein levels in our mouse models. No significant differences were observed, suggesting that results obtained from an immortalized cell line cannot be transposed to what happens in mouse samples. Second, since the lipidic profile could still be altered and be mediated by other targets, we stained WT, PS1KD and PS2KD SH-SY5Y cells using the PhenoVue Nile Red lipid stain. We observed a cytoplasmic staining with inclusions mostly present in PS2KD cells. A significant increase in fluorescence intensity was observed in PS2KD cells compared to WT and PS1KD. Third, in order to understand how the lipid profile could be affected in our mouse models, we stained coronal brain sections using the PhenoVue Nile Red lipid stain. Results showed a specific staining in two subregions of the hippocampal formation: the stratum-lacunosum moleculare (SLM) and the molecular layer of the subiculum (SubM). No significant differences in fluorescence intensity were observed between the different groups in the SLM, but a significant increase was observed in the SubM of PS2 -/- mice compared to WT. Altogether, these data suggest a potential effect of PS2 deletion on the general lipidic profile, which might not be replicated when PS2 is mutated. However, PS2 mutation might have more nuanced influences on the lipidic profile which would require lipidomics analyses to be observed.

Keywords: Alzheimer’s disease, lipids, presenilins

Unravelling the role of Hpcal4 as a potential modifier gene for Rett Syndrome

[1] [1] [1] [1] [2] [2]
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Rett Syndrome (RTT) is a severe neurodevelopmental disorder mainly prevalent among females with 1:10.000 births being affected; it is considered the most frequent cause of profound intellectual disability in females. RTT patients appear to have a normal development up to 6–18 months when the neurological development arrests accompanied by the loss of most early acquired developmental skills, including communication and motor skills. Most cases (90-95%) arise from sporadic mutations within the X-linked gene coding for the methyl-CpG binding protein 2 (MECP2). The protein is ubiquitously expressed and is particularly abundant in brain and associated mutations have profound effects on various neural phenotypes such as impairments in synaptogenesis and neuronal maturation. Besides *Mecp2*, several other genes have been associated with RTT or RTT-like phenotypes and the number of disease-candidate genes has grown over the years. Evidence in literature alongside our RNA-seq data highlighted a downregulation of the Hippocalcin-like 4 (Hpcal4) mRNA in the cerebellum, the hippocampus and the cortex of RTT mouse models lacking *Mecp2,* in addition to a reduction of the protein levels in the cortex of KO animals. The mRNA encodes for a protein that belongs to the visinin-like protein family (VSNL), which are usually involved in the modulation of voltage-gated Ca^{2+} channels, kinase modulation and the Ca^{2+} mediated release of neurotransmitters by vesicles that is relevant to initiate synaptic transmission. However, Hpcal4 functions remain fully undisclosed; indeed, it belongs to the category of Tdark genes, which comprises of protein coding genes with limited or unknown function in literature. Therefore, we aim at clarifying its involvement in neuronal functions in physiological conditions and in RTT and its possible causative link with typically observed RTT neuronal phenotypes. We explored the expression of the protein in the wild type and RTT mouse brain at different time points and found that it is regionally modulated along brain development and deficiently expressed in the symptomatic RTT brain. We additionally analyzed the protein levels in primary neuronal cultures where they were found to be increased during neuronal maturation and reduced in *Mecp2* KO neurons. We further assessed the endogenous expression of the protein in primary hippocampal and cortical neurons and evaluated its colocalization with synaptic markers and determined that the protein is enriched at the level of pre-synaptic compartment. Co-immunoprecipitation of Hpcal4 followed by mass spectrometry analysis in wild type mice cortical lysates revealed several binding interactors that will be validated in future studies, alongside further *in vitro* approaches that will be applied to understand Hpcal4 mechanism of action and dissect its functional role in neurons. These implemented approaches will be fundamental to determine if Hpcal4 can be flagged as a modifier gene for RTT.

Keywords**:** Rett Syndrome, neurodevelopmental disorders, brain

Characterization of Vagus Nerve Electroneurogram (VENG) during acute and chronic kainic acid induced seizures

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Purpose:
Seizures (sz) produce autonomic symptoms, mainly sympathetic but also parasympathetic in origin. Within this context, the vagus nerve (VN) is a key player as it carries information from the different organs to the brain and vice versa. For this reason, exploiting the vagal neural traffic with respect to seizures might offer a novel way to detect seizures and develop a closed-loop Vagus Nerve Stimulation (VNS) system. Therefore, this project aims to develop a Vagus Nerve Electroneurogram (VENG)-based detection algorithm to detect seizures in kainic acid rat (KA) model.
Method:
Anaesthesia was induced using 100mg/kg Ketamine and 7mg/kg Xylazine i.p. and maintained by half concentration of the initial mixture.
Three epidural stainless-steel electrodes were implanted on the frontal cortex ([+]:AP:+2mm, ML:±3mm, [-]:AP:+6mm) to record EEG. A tripolar Micro-Cuff electrode was implanted around the left cervical portion of the VN.
Acute seizure were induced on Six male Wistar rats (296,8±50,2g) under anaesthesia by an injection of KA (0.4µg/0.2µl saline; 0.1µl/min) in the right hippocampus ([RH]:AP:-5.6mm, ML/DV: 4.5mm) and VENG and EEG were recorded for 20 minutes.
Chronic spontaneous seizures were observed post status epilepticus (SE) induction in 2 Wistar rats and surgeries were performed 3 months after SE. VENG and EEG were recorded for 2 weeks.
Vagus nerve activity was characterized on the basis of various parameters such as amplitude modulation or loss of spontaneous rhythmicity.
Results:
23 acute seizures were recorded with a mean duration of 51,21 ± 19 seconds.
Seizures were divided in 2 groups based on VENG changes. 13/23 seizures are characterized by a loss of spontaneous VENG rhythmic activity. 3/13 sz were accompanied by an increase in mean VENG signal amplitude of 56.2 ± 41.2%, while 10/13 sz showed a decrease in mean amplitude of 5 ± 3.4%. The VENG modulation appears in a delay of 25 ± 15 sec after EEG onset.
5 chronic seizures were recorded with a mean duration of 58,8 ± 32,09 seconds. The seizures were characterized by amplitude modulation of the VENG signal, which are currently being analysed.
Conclusion:
Our results show the occurrence of a specific change in VENG activity during acute and chronic KA induced seizures. Therefore, recording of VENG may be useful to detect seizures, which could in the future be used for closed-loop VNS in patients.

Keywords: Epilepsy, Vagus Nerve

Deciphering the Role of Ubiquitination Impairment as a Biomarker and Causative Agent of Tauopathies

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Tauopathies are defined as a group of heterogeneous neurodegenerative disorders, characterized by abnormal Tau protein accumulation in neurons and glial cells. The most common tauopathy is Alzheimer's disease (AD), which is responsible for 70% of cases dementia. The development of tauopathies is a multistep process probably driven by changes in the post-translational modifications (PTMs) of Tau. Unfortunately, for almost all these diseases, no accurate differential diagnostic methods are available which exacerbates the need of identifying biomarkers or treatments for tauopathies. In addition to providing a better understanding of the pathophysiology of tauopathies, the study of PTMs seems well suitable for these aims. According to the literature and previous lab results, ubiquitination appears promising to identify biomarkers allowing the differentiation of tauopathies and also to treat these pathologies. Therefore, the main objective of my thesis is to evaluate the ubiquitination dysregulation in tauopathies and its causal role in protein aggregation. To achieve this goal, we will first investigate the human brain ubiquitinome in several tauopathies (AD, Pick's Disease, Corticobasal degeneration, Progressive Supranuclear Palsy and Frontotemporal lobar degeneration) by mass spectrometry (MS). After identifying specific modifications on proteins (specifically Tau), we will monitor them using targeted MS in the cerebrospinal fluid (CSF) to distinguish between different tauopathies. Moreover, we will assess the potential of the ubiquitination landscape to discriminate between the clinical presentations by correlating the cognitive impairment of patients with the Tau PTMs identified in their CSF. Finally, the role of a potential dysregulation of protein ubiquitination in the development of tauopathies will be investigated in a mouse model and primary neuronal cultures presenting Tau pathology. This may provide novel insights into the beneficial effects of future therapeutic strategies targeting ubiquitination for tauopathies.

Keywords: tauopathies – biomarkers – mass

spectrometry

Investigating crosstalk between intracellular calcium signaling and mitochondrial dynamics

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Parkinson's disease (PD) is the second most common neurodegenerative disease linked to loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Over the last decades, research has identified several hallmarks of PD including mitochondrial dysfunction and impaired calcium homeostasis but the intricate mechanisms underlying these phenomena remain elusive. Lund human mesencephalic (LUHMES) cells, which are human embryonic neuronal precursor cells, have been utilized in several studies to investigate the PD and underlying mechanisms associated with it. We investigate the crosstalk between Ca^{2+} signaling and mitochondrial dynamics by combining live cell imaging and immunostaining techniques. For this aim, LUHMES cells were differentiated into dopaminergic neurons and Ca^{2+} activity was assessed by live cell imaging at two time points of differentiation by addition of external ATP, histamine which stimulate IP_3 mediated Ca^{2+} signaling and FCCP to access the effect of morphological changes on Ca^{2+} signaling. After imaging acquisitions, cells were analysed for inter-spike intervals, spike width and averaged Ca^{2+} concentration in the cytoplasm. To check the morphology of the mitochondria, cells at day 5 were treated with ATP and histamine and FCCP as a positive control and stained with TOM20. Our analysis shows a relationship between calcium activity induced by ATP and histamine, and morphological changes in mitochondrial. Furthermore, the results also indicate a higher activity of Ca^{2+} channels in post mitotic dopaminergic neurons as compared to the immature cells. Overall, our study on Ca^{2+} live cell imaging revealed novel aspects, which can be used as a model to further understand Ca^{2+} signaling in dopaminergic neurons.

Keywords: Calcium signaling, Mitochondrial dynamics, Parkinson's disease

Interplay of autophagy and metabolic rewiring in the context of neurodegeneration and synaptic physiology

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Maintaining regular brain activity requires a tight regulation of the metabolites and energy consumption [1, 2]. To meet this energy demand, an efficient nutrient recycling procedure is needed. The efficiency of (macro)autophagy, one of the most important organelle and protein degradation systems of the cell is decreasing with age and is connected to many neurodegenerative diseases as Alzheimer's and Parkinson's diseases or ALS [3, 4]. Creating Autophagy related protein 5 knockout (ATG5 KO), only affecting progenitor cells, showed to lead to severe neurodegeneration in multiple studies [5-7]. However, when ATG5 was deleted in post-mitotic neurons no loss of neurons could be observed in the cortex, but degeneration of synapses could not be prevented and also Purkinje cells were observed to be vulnerable to autophagy loss [8-10]. This leads to the question whether autophagy plays a brain region-specific role in neuronal survival?

Various previous experiments in cortical neurons and Purkinje cells showed significant differences in metabolic pathways, especially hinting towards changes in β-oxidation and pentose phosphate pathway (PPP) under autophagy deficient conditions. To further investigate these differences, the aim of this study is to understand the role of autophagy in metabolic rewiring in the cortex and its role in preventing neurodegeneration. More specific we want to investigate whether upregulation of β-oxidation and/or the PPP help cortical neurons to survive under autophagy deficient conditions? And does it have a detrimental effect on synaptic physiology?

Little is known about β-oxidation, the mitochondrial degradation pathway of fatty acids in neurons, but it was shown to be important for energy production and preventing fatty acid toxicity in astrocytes [11, 12] and been linked to autophagy in various cell types [13, 14]. Fatty acids are transported over the mitochondrial membrane using the carnitine shuttle, with its key enzyme Cpt1a, a process which can be inhibited by the drug etomoxir [15, 16]. Whereas the PPP serves several important functions in the cell, as the production of reduced NADPH for the regeneration of antioxidants or the production of ribose-5-phosphate [17]. Inhibition of G6PD, the rate limiting enzyme of PPP increases autophagy [18] and that overexpression of G6PD is neuroprotective in neuronal cell culture [19, 20]. Preliminary data, using GCaMP7f and electric stimulation as readout for synaptic firing show that neuronal activity is decreased in ATG5 KO cells, but especially etomoxir-treated KO neurons showed a strong decrease in neuronal activity, suggesting that autophagy-deficient cortical neurons might switch to β-oxidation for energy demands, a hypothesis to be investigated further. Further experiments will follow, using a new ATG5/Cpt1a KO line to examine β-oxidation and knockdown/ overexpression of G6PD to investigate the PPP under autophagy-deficient conditions.

Keywords: autophagy, β-Oxidation, neurons

Lysosomal alterations in Charcot-Marie-Tooth disease type 1A

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INTRODUCTION: Charcot-Marie-Marie Tooth disease type 1 (CMT1) is the most common hereditary demyelinating peripheral nerve disease occurring in 1 in 2500 people worldwide. Its predominant subtype is CMT1A and is caused by a duplication of the peripheral myelin protein 22 (PMP22). This aggregation-prone protein is mainly expressed by Schwann cells, the myelinating cells of the peripheral nervous system. Previous research demonstrates that myelinating cells are in particular sensitive for lysosomal stress, making it presumable that overexpression of PMP22 interferes with lysosomal function. Nevertheless, the role of lysosomal dysfunction in defective myelinating cells and CMT1A remains poorly understood. Here, we monitored lysosomal alterations in a the C3 mouse model for CMT1A and confirm our results in CMT1A patient-derived human induced pluripotent stem cell derived Schwann cell precursors (SCP).

METHODS & RESUlTS:

Sciatic nerves were isolated from 4-8-week-old C3 and WT mice to monitor protein levels of the lysosomal marker LAMP1 and lysosomal enzymes cathepsin B and D (CTB and CTD) using western blot. This indicated a significant increase in lysosomal amount and lysosomal enzymes in CMT1A ($p=0.008$, $p=0.003$ and $p=0.045$, respectively). These results were confirmed using immunostainings in CMT1A sciatic nerves (LAMP1 $p=0.0007$, CTD $p=0.0025$, CTB $p=0.0043$). In addition, primary Schwann cells were isolated from C3 and WT mice. LAMP1, CTB and CTD protein levels were visualized using immunostainings, showing a significant increase in CMT1A Schwann cells ($p<0.0001$, $p<0.0001$, $p=0.0087$, respectively). Additionally, the activity of CTB was significantly elevated in primary CMT1A Schwann cells ($p<0.0001$). CMT1A Patient-derived SCP confirmed these data in primary cells, showing significantly higher LAMP1, CTD and CTB levels compared to their isogenic controls ($p<0.0001$, $p>0.0001$n $p=0.0207$ respectively), using immunostainings.

CONCLUSIONS: We could demonstrate a significant increase in lysosomal numbers, lysosomal enzymes and their activity in the peripheral nerves of CMT1A mice and primary CMT1A Schwann cells. In addition, these data could be recapitulated in human CMT1A patient iPSC-derived Schwann cell precursors. These results indicate significant changes in lysosomes and their enzymes in CMT1A disease. Nevertheless, further research is necessary to further explore the mechanism and consequences of these lysosomal changes, and their possible use as a therapeutic target.

Keywords: Schwann cells, Charcot-Marie-Tooth disease, Lysosomes

Deciphering the Mode of Action of Small anti-Alzheimer Drugs on the Amyloid precursor protein metabolism and Aβ peptide production

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As part of a search for small molecules for therapeutic purposes against Alzheimer's disease (AD), the collaborative efforts of Professor Melnyk and Dr. Buée have yielded five distinctive families of molecules through cellular phenotypic selection. Several “lead” molecules within these families have demonstrated efficacy on the two lesion processes that define Alzheimer's disease (AD), namely, amyloid deposits and neurofibrillary degeneration (NFD). Moreover, in vivo administration of these molecules has revealed anti-inflammatory properties, manifesting as a reduction of astrogliosis and neuroinflammation and recovery of cognitive functions observed in transgenic models of amyloid deposits (APPxPS1) or NFD (Thy-Tau22). These results then suggest that a same compound can act on both AD lesion processes. However, despite the promising in vivo therapeutic effects, critical aspects of these molecules, including their biological target, mechanism of action, and mode of action, remain undetermined.

To gain deeper insights into the mode of action of these anti-Alzheimer drugs, collaboration with the chemistry team led by Professor Patricia Melnyk has led to the selection of six hit compounds from the five families. The selected compounds (CME22-128, CME 22-176, CME 22-149, FD15, RPEL88, and PEL24-199) were chosen based on their pharmacological impact on amyloid precursor protein (APP) metabolism and Tau aggregation. Experimental assessments aimed to determine the extent to which these compounds reduce Aβ peptide production involving the use of SY5Y-APP695 cells stably expressing human APP and inducible SY5Y-C99 expressing cells. The first model helps assess the effect on Aβ production through the successive cleavage of APP by β- and γ-secretases, while the latter expresses APP fragments (C99 fragments) precluding the β-secretase cleavage of APP. This model serves to determine the impact on Aβ production downstream of β-secretase cleavage and therefore to determine at which level of APP metabolism our drugs are reducing the amount Aβ peptide produced

Our current ongoing results show that all five drugs suppress Aβ peptide production in both SY5Y-APPC695 and SY5Y-C99 cellular systems, suggesting a mode of action either at the level of γ-secretase or a Aβ degradation.

Further investigations on autophagy and lysosome activity were carried out to study whether they could contribute to the reduction of Aβ through a degradation mechanism. An increase in p62/SQSTM1 expression and a slight elevation in the conversion of LC3B-I to LC3B-II were shown. The results obtained following drug treatment differ from those obtained with autophagy inhibitors, such as chloroquine and bafilomycin A1. These observations collectively indicate the potential involvement of an autophagic and lysosomal-dependent mechanism may contribute to the observed reduction in Aβ peptide levels.

While γ-secretase activity remained unaffected by our drugs, as evidenced by preserved ΔENotch cleavage in ICD, ongoing research suggests that drugs from these families exert their effects through a complex, lysosomal and autophagic-dependent mechanism. These findings highlight the necessity for comprehensive investigations to understand the precise mode of action underlying the anti-Alzheimer effects elicited by the drugs. These results demonstrate the importance of these compounds in the search for targeted treatments for Alzheimer's disease, marking an important step toward understanding AD pathology and advancing viable treatment modalities.

Keywords: Alzheimer's disease, autophagy, drug development

Spatiotemporal analysis of midbrain dopaminergic neurons in an aav-mediated αlpha-synuclein parkinson's mouse model

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Key characteristics of Parkinson's Disease (PD) include the progressive and selective degeneration of dopaminergic neurons (DAns) in the substantia nigra (SN) and intraneuronal α-Synuclein inclusions known as Lewy bodies. DAns can be categorized based on various factors such as brain location, expression profile, and physiological function. A major subtype of nigral DAns expressing aldehyde dehydrogenase 1 family A1 (ALDH1A1) has been identified as selectively vulnerable in PD brains and is over-represented in pathways underpinning vulnerability in PD models. However, several findings postulate a complex molecular characterization of vulnerability that has not yet been delineated. This study characterizes the transcriptome of ALDH1A1+ and ALDH1A1- DAns of an AAV-mediated mouse model of α-Synuclein pathology.

Mice received intra-nigral injections of AAV-expressing human-α-Synuclein or GFP. 100 slices of 12µm thickness were sectioned from the midbrain at 3- and 8-weeks post-injection, corresponding with pre- and post-cell death. Neuroanatomical regions were delineated and ALDH1A1+/- DAns were identified using immunofluorescence. Spatially-resolved transcriptomics was performed on midbrain ALDH1A1+/- DAns using NanoString's GeoMx Digital Spatial Profiler. Bioinformatics analyses were performed in R.

We demonstrated diffuse α-Synuclein pathology in the midbrain and increased SNCA expression at all timepoints. Significant DAN loss was observed in the SN at 8 weeks, with no DAN loss evident in the ventral tegmental area (VTA). We did not measure differences in vulnerability between nigral ALDH1A1+ and ALDH1A1- DANs. Differential expression analysis identified distinct transcriptomic signatures of ALDH1A1+/- subtypes at early and late timepoints and indicated that α-Synuclein pathology may reduce transcriptional differences. Enrichment analysis identified critical pathways associated with each subtype and shed light on the functional impact of α-Synuclein pathology in these cells. We here provide a comprehensive spatial and temporal characterization of murine midbrain ALDH1A1+ vs ALDH1A1- dopaminergic neurons and the effect of α-Synuclein pathology on these subtypes.

Keywords: Spatial-transcriptomics, cell-type-specific vulnerability, Parkinson's disease

The effects of PMP22 overexpression on endoplasmic reticulum stress in Charcot-Marie-Tooth disease type 1A

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INTRODUCTION: Charcot-Marie-Tooth (CMT) disease is an inherited peripheral neuropathy, affecting 1 in 2,500 people worldwide. The most common form of the disease, **CMT1A**, is predominantly demyelinating and is caused by a ***Peripheral Myelin Protein 22* (*PMP22*)** gene duplication. PMP22 is an aggregation-prone intrinsic membrane protein of the myelin sheath mainly produced by Schwann cells (SC). It is **unknown** how the overexpression of PMP22 contributes to the abnormal myelin sheath structure and dysfunction observed in CMT1A. Hence, there is no cure available to date.

We **hypothesize** that the overexpression of PMP22 leads to an overload of the protein in the **endoplasmic reticulum (ER)**, inducing ER stress and activating the unfolded protein response (UPR). Therefore, we **aim** to investigate the effect of PMP22 overproduction and aggregation in the ER on the UPR in CMT1A Schwann cells.

METHODS & RESULTS: Nerve tissue and SC were isolated from wild-type (WT) and C3-PMP22 mice, an animal model for CMT1A. Furthermore, we used a human patient-in-a-dish model of CMT1A patient-derived human induced pluripotent stem cells (hiPSCs) and their isogenic controls differentiated towards Schwann cell precursors (hiPSC-SCP).

Protein ubiquitination was observed via immunostainings in primary murine C3 SC. Furthermore, the ER is more densely organized in C3 SC and CMT1A mice as observed using calnexin immunofluorescence stainings. Additionally, we confirmed a correlation between protein levels of the ER chaperone calnexin protein and PMP22 in CMT1A hiPSC-SCP. Lastly, compared to controls, ER stress sensor ratios of phosphorylated protein kinase R-like ER kinase (P-PERK)/PERK were upregulated in CMT hiPSC.

CONCLUSION: We conclude that ER stress is present in CMT1A SC and tissue as confirmed by immunofluorescence and western blot analyses. Future experiments are necessary to indicate how this affects SC myelination, providing important insights into CMT1A and other neurodegenerative, demyelinating, and PMP22-related diseases.

Keywords: Charcot-Marie-Tooth disease type 1 A, Schwann cells, protein aggregation

Effects of fullerenol-based antioxidants, transplantation therapy and newly FDA-approved Lecanemab on behavioral, molecular and histological hallmarks of Alzheimer's disease in aged APP/PS1 female mice

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Abstract

Background. The study was aimed at the investigation of new molecular mechanisms of neurodegeneration underlying Alzheimer's disorder (AD) and potential beneficial effects of novel anti-oxidants, as well as of a new transplantational therapy, in comparison with those of recently FDA-approved Lecanemab. Given that oxidative stress and neuroinflammation are considered as key pathological processes underlying amyloid plaque formation and neuronal death, we studied the effects of chronic dosing with anti-oxidants fullerene and fullerenol, as well as effects of 'Neuro-Cells' (NC)unmanipulated human stem cell preparation of Mesenchimal stem cells (MSC) and Hemopoetic stem cells (HSC) in a well-established animal model of AD, APP/PS1 transgenic mice. As a reference treatment, Lecanemab whose administration diminished amyloid plaque formation was employed.

Methods. We used female APP/PS1 mice and their wild-type (WT) littermates that were subjected either to **(a)** 10-month dosing with fullerenol with drinking water; **(b)** 10-month dosing with fulleren with a diet; **(c)** single administration of 500 000 NC to cysterna magna; **(d)** double i.v. injection of Lecanemab with 2 week interval, **(e)** a combination of NC and Lecanemab treatment, or **(f)** no treatment. Mice were about 1 year old at the end of the study; behavioral measures of locomotion, anxiety, and learning were investigated starting 48 h after the last Lecanemab administration. Brains from all groups of mice were harvested for amyloid beta Congo staining, 6E10 antibody, DAPI and GFAP in the cortex, hippocampus and thalamus. Gene expression of the markers of neurons, astroglia and inflammation were studied in the hippocampus and prefrontal cortex. Deep learning methods were applied to study the density of amyloid plaques of various sizes: <100 nm, 100-200 nm, 200-500 nm and >500 nm. **Results. W**e found profound cognitive deficits of APP/PS1 mice and increased anxiety accompanied by massive plaque formation and pro-inflammatory changes in the brain. These abnormalities were ameliorated by the use of the treatments, from which fullerenol and a combination of NC were particularly effective.

Conclusions. Our study identified new correlates of AD-like pathology in APP/PS1 mice and proposes the use of fullerenol, and NC transplantational therapy as potential new treatment of AD.

Keywords: APP/PS1-transgenic mice, Neuro-Cells, Lecanemab, fullerene, fullerenol

Development of an *ex vivo* model to study the regional vulnerability in tauopathies

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Tauopathies are a heterogeneous group of neurodegenerative diseases whose common feature is the filamentous inclusion of aggregated and abnormally phosphorylated tau protein in brain cells. Experimental evidence suggests that pathological species of tau may act as seeds leading to *de novo* aggregation of endogenous/physiological tau. It is also proposed that in some tauopathies, including Alzheimer disease (AD), tau seeds can be transferred from cell to cell and spread between different brain regions. This would explain the spatial and temporal progression of tau pathology in some tauopathies such as AD. However, the existence of connections between two regions is not always sufficient to explain the spread of tau pathology, suggesting that some brain cells/regions are more vulnerable than others.

In this scientific context, the objective of this study is to develop an *ex vivo* model to assess the vulnerability of certain brain regions to develop but also to propagate distinct tau pathologies.

To do so, we prepare organotypic slice cultures of mouse brain regions expressing mutated human tau protein onto which we apply human brain homogenates prepared from patients with tauopathies or control subjects. We monitor the potentiation of tau lesions by immunostaining and the *de novo* production of tau seeds by FRET. In parallel, we assess their extracellular activity using a microelectrode array system.

After setting the parameters to achieve reproducible potentiation of tau pathology, we were able to potentiate an abnormal conformation of tau protein in the hippocampus of transgenic mice expressing mutated human tau protein by adding brain homogenate from an AD patient. We then expect to observe variable effects between brain regions exposed to the same experimental conditions.

The setting of such an *ex vivo* system will make it possible to assess the intrinsic capacity of different brain regions to develop and propagate different tau pathologies.

Keywords: Tau protein, Organotypic brain slice culture, regional vulnerability

Human nasal beta-amyloid 42 has a diagnostic value in discriminating Alzheimer's disease

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Background: The key in Alzheimer's disease (AD) therapy is a timely and accurate diagnosis for prompt drug intervention. However, due to the high cost and invasiveness of conventional biomarker analyses, including brain positron emission tomography (PET) imaging and cerebrospinal fluid (CSF)-based assays, easy accessibility to these screening tests is often hindered. There is, therefore, a great need to develop a more accessible biomarker screening test using less invasive and cost-effective peripheral body fluid biomarkers. Previous studies examined the non-quantitative expression of beta-amyloid (Aβ) in normal and AD patients' nasal discharge fluid. They identified higher expression of oligomeric Aβ in AD patients, showing a correlation with cognitive decline. However, the quantitative measurements of nasal Aβ42 levels, including the full AD continuum, remain unknown. Here, we assessed whether quantified human nasal Aβ42 levels have diagnostic performance and have the potential to be utilized as a biological risk factor in AD.

Method: 161 subjects (cognitively normal (CN), n=32; preclinical, n=29; mild cognitive impairment (MCI), n=73; AD, n=27) underwent neuropsychological battery tests, including Mini-Mental State Examination (MMSE), Clinical Dementia Rate (CDR), and Global Deterioration Scale (GDS), and amyloid-PET scans (18F-Flutemetamol). Their nasal discharge samples were collected, and nasal Aβ42 levels were measured via enzyme-linked immunosorbent assay (ELISA).

Result: We found that the second-highest quartile (Q3) group of nasal Aβ42 constituted the majority of patients with AD diagnosis (p=0.036). The Q3 group also outnumbered the other groups in the most cognitively impaired subjects in all three neuropsychological battery tests (p=0.008, p=0.037, p=0.023). Next we also found that subjects in the Q3 group exhibited most impaired cognitive function in all three neuropsychological battery tests with strong significance. When we examined the amyloid-PET SUVR values, subjects with PET positive showed the highest SUVR.

Conclusion: Quantified nasal Aβ42 is strongly associated with cognition measurements. Nasal Aβ42 suggests the possibility for discriminating AD from non-AD.

Keywords: Alzheimer's disease / olfactory dysfunction / biomarker

Computational modelling of cAMP signalling in the context of Alzheimer's disease

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Cyclic adenosine monophosphate (cAMP) serves as a versatile signalling molecule with distinct cellular effects, the precise regulation of which is crucial for maintaining cellular homeostasis. Aberrations in cAMP signalling, associated with various diseases such as Alzheimer's disease, spinal cord injury and multiple sclerosis, underscoring the need for a deeper understanding of the underlying regulatory mechanisms. Among these, phosphodiesterase 4 (PDE4) enzymes play a pivotal role in modulating cAMP signalling through differential isoform-specific feedback mechanisms.

Via computational modelling, we investigated how feedback mechanisms on different PDE4 isoform types contribute to the control of cAMP signalling. Simulations utilizing ordinary differential equations to describe cAMP dynamics revealed that long PDE4 isoforms exerted profound control over oscillatory cAMP signalling in contrast to a single cAMP input pulse.

Our findings emphasize the necessity of considering the isoform specificity of PDE4 enzymes in both computational and experimental studies. These insights contribute to the refinement of PDE4-targeted therapeutic strategies in conditions where cAMP signalling is aberrant. Future perspectives include exploring PDE4/cAMP signalling in specific cellular compartments or cell types through adaptations of the presented model. Additionally, the model facilitates the examination of PDE4 inhibitors with varying affinities to different isoform types, providing an interesting future perspective into drug design strategies targeting overall cAMP signalling.

Keywords: max. 3 words cAMP signalling; PDE4 inhibition;

computational modelling

Forever young – the epigenetic clock of OPCs

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With 2.8 million reported cases in 2020, multiple sclerosis (MS) is the most prevalent demyelinating neurodegenerative disorder affecting the central nervous system (CNS). Demyelination, involving the loss of the protective nerve insulation, disrupts nerve signal transmission and leads to symptoms such as fatigue, muscle weakness and memory loss. Typically, demyelination is followed by the recruitment of oligodendrocyte precursor cells (OPCs), which subsequently undergo differentiation into oligodendrocytes (OLs) capable of remyelination. Although it is established that remyelination failure in progressive MS results from premature OPC aging and subsequent differentiation failure, the precise mechanism behind this premature aging process remains elusive. Our approach involves comparing OPCs derived from aged mice with those from newborn mice, anticipating the identification of differentially expressed genes (DEGs) associated with aging. Concurrently, we will investigate the DNA methylation patterns of genes related to the aging process, recognizing the pivotal role of DNA methylation in regulating gene expression, and its established connections with aging and the progression of MS. We expect to identify differentially methylated regions in the promotors of important senescence genes such as cdkn1/2 and p53, as these genes have previously been linked to OPC aging. For DEGs recognized by altered DNA methylation patterns, we will employ CRISPR/dCas9 technology in combination with methylating (DNMT3a) or demethylating (TET1) enzymes. With this gene editing tool we aim to restore the methylation patterns in aged OPCs with those found in newborn mice. In this way, the ultimate aim is to rejuvenate the aged OPCs, restoring their capability to differentiate into OLs that can effectively repair demyelinated lesions. Given that demyelination is the primary cause of MS disease progression and with no current remyelination-based therapies available, our aim is to apply this research in future MS studies.

Keywords: Senescence, DNA methylation, oligodendrocyte precursor cell

Wrapping up Alzheimer's disease: Targeting myelination by editing DNA methylation

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Accumulating evidence implicates oligodendrocyte dysfunction and myelin degeneration in Alzheimer's disease (AD). Oligodendrocytes are responsible for producing lipid-rich myelin around the neuronal axons. Myelin facilitates signal transduction and acts as a protective barrier. In AD, there is an excessive loss of myelin and this loss strongly correlates with cognitive decline. Both oligodendrocyte development and myelination are regulated by epigenetic modifications, including DNA methylation. We hypothesise methylation patterns of oligodendroglial cells to be altered in AD, thereby blocking remyelination and contributing to myelin degeneration. Although differential methylation patterns have been observed in AD, oligodendrocyte-specific patterns have yet to be established. We therefore aim to identify genetic positions and regions that are differentially methylated in oligodendrocytes derived from post-mortem brain tissue of AD and mild cognitive impairment patients as compared to non-neurological controls. Similarly, we will compare the epigenetic profiles of APP/PSEN1 - a well-established AD mouse model - and wildtype mice. Based on gene ontology and pathway analyses, we will assess the relevance of the identified genetic targets in the context of myelination. The five most significant myelin-associated targets will be investigated more thoroughly. We will use laser capture microdissection to isolate oligodendrocytes from post-mortem brain sections and compare the methylation status of the five targets in oligodendrocytes in close proximity (100 μm radius) and distant to amyloid beta. The clinical relevance of the methylation patterns will be assessed by including the subject's score on the mini mental state exam in our analyses. To directly assess the biological effects of (de)methylation of the targets, we will epigenetically edit induced pluripotent stem cell-derived oligodendrocytes using the CRISPR-deadCas9 system fused to a methylating (DNMT3a) or demethylating (TET1) enzyme.

Oligodendrocyte morphology and functioning will be characterised by assessing the gene expression of various oligodendrocyte markers, oligodendrocyte-neuron interactions in co-cultures, and the myelination capacity in a microfiber assay. Altogether, this research will allow us to elucidate the role of epigenetics in the white matter loss in Alzheimer's disease. The findings may provide new avenues for therapeutic approaches.

Keywords: Oligodendrocytes, epigenetics, Alzheimer's disease

Large and small extracellular vesicles from the brain-derived fluid of AD patients reveal a pathological signature and a differential implication in tau nucleation

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Introduction Alzheimer's disease (AD) is a neurodegenerative disease characterized by neurofibrillary degeneration following a spatio-temporal progression in the brain. This could be linked to a prion-like propagation mechanism where seed-competent (pathological) tau proteins spread from one cell to another. Extracellular vesicles (EVs) which are unique intercellular lipid delivery shuttles with peculiar transmembrane proteins, may be implied in this spatio-temporal progression. After numerous animal studies, recently, we and others demonstrated that EVs from brain-derived fluid of AD patients have both an *in vitro* and *in vivo* tau seeding capacity. Based on the rich diversity of EVs, we now aim to separate EVs into subpopulations with subsequent proteomic profiling to gain insights into AD-specific EV pathophysiological signature. Further, we assessed the implication of these EVs subpopulations in tau seeding.

Method The brain-derived fluid is obtained by enzymatic dissociation of AD and non-demented control brain extracts from the Lille Neurobank. Size exclusion chromatography (SEC) allows EVs purification from soluble contaminants and progressive centrifugation steps enable separation of large EVs (LEVs: >150nm) and small EVs (SEVs: 10-150nm). These were characterized using NTA, electron microscopy and HPLC/MSMS. Their seeding capacity was studied using a FRET based biosensor cell assay.

Results A major impact on EV protein yield was observed depending on the used enzyme for the brain dissociation; papain versus collagenase. Collagenase allows a clear downstream separation of LEVs and SEVs subpopulations characterized with specific expressed proteins and numerous subpopulationenriched transmembrane proteins. Interestingly, LEVs and SEVs of AD patients indicate pathogenic aspects underlying AD, such as integrin signalling, inflammation and genes of the GWAS. Further, we have shown an increased presence of glial secreted EVs in Ad patients. Finally, *in vitro* studies have shown a higher tau seeding capacity for LEVs compared to SEVs.

Conclusion Collagenase3 brain dissociation leads to a higher protein yield and higher EV integrity. Hence, being most suited for downstream proteomic and functional analysis. Both LEVs and SEVs revealed an AD-specific pathophysiological signature, although LEVs show a higher *in vitro* seeding capacity than SEVs. At this day, the seeding capacity of LEVs and SEVs will be analysed *in vivo* by injection in transgenic mice.

Keywords: Alzheimer's Disease, Prion-like tau propagation, Extracellular vesicles

Specific dimeric orientations in the amyloid pathology of Alzheimer's disease

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Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by the accumulation of amyloid peptides (Aβ) within the brain. This buildup of Aβ peptides sets off a pathological cascade of events that eventually leads to cognitive decline and dementia witnessed in AD. Even if the accumulation of amyloid plaques does not seem to correlate with the onset of clinical symptoms, recent research has unveiled a paradigm shift, spotlighting soluble Aβ oligomers as the principal instigators of cellular toxicity, inflammation, and oxidative stress in the progression of AD. However, the molecular structure of these toxic Aβ species and their link to the development of the disease remain poorly understood.

During this project, we seek to gain insights regarding these toxic Aβ species, their molecular structure and their intricate connection to the onset and progression of Alzheimer's disease. Therefore, we will focus on factors influencing the formation of these toxic oligomers, such as the gamma-secretase complex. This complex, responsible for the production of Aβ, possesses catalytic subunits presenilin 1 or presenilin 2. Notably, PS1-dependent cleavage predominantly produces Aβ40 extracellularly, while PS2-dependent gamma-secretase is linked to endocytic compartments, generating an intracellular pool of Aβ42 that is more prone to aggregation, forming toxic oligomers.

Additionally, the formation of these toxic oligomers may be influenced by the biophysical parameters of the Aβ precursor, CTF-B, or C99. Recent findings from the hosting team indicate that specific dimeric conformations of the amyloidogenic APP C-terminal fragment (CTF-β or C99) regulate the production and formation of pathogenic Aβ oligomers. Moreover, the lipid composition of the membrane was found to influence the structure and stability of C99, highlighting the complexity of the Aβ production process. Finally, when supposing that these specific C99 dimeric conformations shape the biophysical properties of Aβ42, early findings suggest that specific dimeric Aβ42 conformations could act as barriers to the formation of toxic oligomers. This revelation opens a promising avenue for therapeutic intervention, suggesting that certain Aβ42 dimers could potentially inhibit the development of the very species driving the neurodegenerative cascade.

In summary, this research project aims to unravel the intricate mechanisms behind the formation of pathogenic Aβ assemblies, focusing on the role of APP dimerization and specific dimeric conformations in the production of Aβ peptides, with the ultimate goal of developing innovative therapeutic strategies for Alzheimer's Disease.

Keywords: Alzheimer's disease, Aβ oligomers, Dimeric C99 conformations

Extracellular vesicle-associated cholesterol dictates the regenerative functions of macrophages in the brain

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Failure of remyelination underlies the progressive nature of demyelinating diseases. Recently, we and others demonstrated that macrophages are abundantly present in multiple sclerosis (MS) lesions and play a pivotal role in disease progression and resolution. To date, however, it remains largely unknown how macrophages contribute to CNS repair. Here, we demonstrate that extracellular vesicles (EVs) secreted by repairassociated macrophages promote oligodendrocyte precursor cell (OPC) differentiation *in vitro* and enhance remyelination in the cerebellar brain slice and cuprizone models. Additionally, by applying cholesterol depleting and enrichment strategies, we identify that the pro-regenerative impact of EVs released by repair-associated macrophage relies on cholesterol abundance, but did not depend on liver X receptor activation prior to OPC maturation. Altogether, our findings suggest that EV-associated cholesterol is a driving factor in the regenerative impact of lesional macrophages on OPC maturation and remyelination, potentially having broad implications for diagnostic and therapeutic strategies aimed at promoting remyelination.

Keywords: Remyelination, cholesterol, macrophage-derived extracellular vesicles

Effects of adenosine A 2A receptor astrocytic upregulation in the mouse hippocampus

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Alzheimer's disease (AD) is notably characterized by the intraneuronal aggregation of tau proteins which play an important role in synaptic dysfunctions and memory decline. Studies have reported that chronic caffeine consumption reduces AD risk and cognitive deficits. These protective effects would be ascribed to the blockade of adenosine A 2A receptors (A 2A Rs) which are pathologically upregulated in the hippocampus of patients with AD. This upregulation is observed in the astrocytes - which are essential cells for brain homeostasis- and has been correlated with the pathology development and associated cognitive deficits.

However, the mechanisms underlying the link between astrocytic A 2A R upregulation and memory deficits remain unclear. To uncover the impact of astrocytic A 2A R upregulation, we intrahippocampally injected an AAV2/9 virus expressing A 2A R (AAV-A2A), or GFP as control (AAV-GFP), under a GFAabc1d astrocyte-specific promoter, in 2m-old C57Bl6/J mice. We evaluated consequences in term of spatial memory performance, response to hippocampal neural networks using DREADDs as well as astrocyte reactivity, morphology and transcriptome.

Our data show that A 2A R overexpression in hippocampal astrocytes impairs short-term spatial memory (Y-Maze task) and long-term spatial learning (Barnes Maze task). At the network level, thanks to the DREADD approach, we observed an enhanced neuronal excitability in animals injected with the AAV-A2A as compared to the control GFP group, characterized by a higher immediate early gene response. These changes were associated with deep alterations of astrocyte reactivity, morphology and transcriptome. These results therefore demonstrate that upregulation of A 2A R in hippocampal astrocytes, as seen in the brains of AD patients, is sufficient to alter astrocytic phenotype, neuronal response and memory.

To determine the pathophysiological impact of A 2A R astrocytic upregulation, we are now currently determining the effects of the latter at an early stage using a mouse model of AD-like tauopathy (Thy-Tau22). Our first results indicate that A 2A R dysregulation in astrocytes potentiates Tau-induced memory deficits. Impact on brain lesions as well as on glial response are currently under investigation. We expect to uncover mechanisms linking astrocyte dysfunction to the evolution of tauopathy and provide additional proof-of-concept that targeting A 2A R is of therapeutical importance in AD and tauopathies.

Reactive Astrocytes from patients affected by Amyotrophic Lateral Sclerosis: how mGlu5 receptor modulation affects pathological features

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BACKGROUND. Amyotrophic Lateral Sclerosis (ALS) is a multifactorial non-cell-autonomous neurodegenerative disease, characterized by motor neurons (MNs) death. The metabotropic glutamate receptor type 5 (mGluR5) plays a key role in modulating glutamate excitotoxicity and astrocyte reactivity as major cause of MNs loss. We provided *in-vitro* and *in-vivo* evidence showing that genetic ablation or the pharmacological modulation of mGluR5 by the selective negative allosteric modulator CTEP positively affects the reactive phenotype and neurotoxicity of ALS astrocytes and significantly improved the life span and disease progression in $SOD1^{G93A}$ ALS mice. Here we investigated *in-vitro* the impact of mGluR5 modulation by CTEP on *i-Astrocytes* differentiated from inducible neural progenitor cells (iNPCs) of ALS patients and control donors.

RESULTS. *In-vitro* pharmacological modulation with CTEP did not alter the mGluR5 total expression in *i-Astrocytes*. Confocal microscopy, immunohistochemical experiments and RT-qPCR analyses showed that *in-vitro* exposure to 100nM CTEP reduced the over-expression of markers linked to aberrant activation and neuroinflammation (GFAP, S100β, C3, NLRP3), in *i-Astrocytes* carrying the C9orf72 and SOD^{A4V} ALS-mutations vs. untreated cells. Of note, the phenotype shift induced by mGluR5 negative modulation, was accompanied by increased Nrf2 nuclear translocation, enhancement of the antioxidant enzymes activity (glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, catalase), reduced ROS and malondialdehyde accumulation, compared to untreated *i-Astrocytes* and control cells. Preliminary results also showed that CTEP hampers the excessive intracellular calcium mobilization in ALS reactive *i-Astrocytes*

CONCLUSIONS. We here show that the *in-vitro* pharmacological negative modulation of the mGluR5 by CTEP positively affects the reactive phenotype of human-derived *i-Astrocytes* from C9orf72 and SOD^{A4V} ALS patients, mainly by ameliorating the oxidative stress response of these cells. These data extend our previous results in the $SOD1^{G93A}$ mouse model, thus further encouraging a potential translational application of mGluR5 modulators in clinical trials.

Keywords: ALS, human astrocytes, mGluR5

Exploring proteomic targets in brain tissue of mice with cognitive impairment induced by carbon nanoparticle exposure

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Air pollution, in particularly exposure to airborne particulate matter, has been implicated in various adverse health effects, including neurodegenerative impairment(s). recently, we noted the transfer of carbon nanoparticles (CNP), a component of ultrafine particulate matter, into the brain.

This study aims to elucidate proteomic alterations in the brain tissue and blood of mice exhibiting cognitive deficits following exposure to carbon nanoparticles (CNP). Pregnant wildtype C57BL/6J mice were either sham-exposed (HEPA-filtered air) or CNP-exposed (440 µg/m3 pure aerosolized carbon nanoparticles) for four hours per day for four consecutive days per exposure period. The mice and their offspring were further divided into four groups, being i) sham, ii) only prenatally, iii) only postnatally, and iv) both pre- and postnatally exposed. The mice of the only prenatally, only postnatally, and both pre- and postnatally exposed groups were re-exposed to CNP in the adult phase to simulate chronic exposure.

Cognitive and social-emotional performance was assessed using a battery of behavioral tests, revealing significant impairments in spatial memory and increased anxiety-like behavior in exposed mice compared to controls. Subsequently, proteomic analyses of 96 protein targets (Target 96 Mouse Exploratory panel, Olink® Proteomics) were conducted on both brain tissue lysates (n=16 per group) and blood samples (n=6 per group). Proteomic profiling identified a several of differentially expressed proteins associated to CNP's exposure.

In conclusion, this study contributes to our understanding of the brain proteomic landscape associated with cognitive impairment following exposure to CNPs, shedding light on potential molecular pathways affected by air pollution. These findings may have broader implications for elucidating the mechanisms linking air pollution to neurodevelopment and identifying biomarkers for at-risk populations.

Keywords: Ultrafine particulate matter, Proteomics, behavioral development

Preliminary Results of a Longitudinal Vagus Nerve Stimulation Study: Effects on Laryngeal Motor Evoked Potentials and EEG Synchronization

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Rationale

Previous studies have shown that Vagus Nerve Stimulation (VNS) induces acute electroencephalogram (EEG) desynchronization. Laryngeal Motor Evoked Potentials (LMEPs) are markers of efferent A-fiber activation and can be recorded using two surface electrodes on the ventral surface neck. Currently no reliable biomarker for optimizing VNS treatment exists. In addition, there is no information available on how (i) vagus nerve recuperates after surgery, (ii) VNS-induced EEG desynchronization evolves postoperatively, and (iii) LMEPS change after the implantation. This study aims at assessing longitudinally efferent and afferent markers of VNS action.

Methods

Adult patients with drug-resistant epilepsy, candidates to VNS implantation, were prospectively recruited for the current study. EEG was recorded 1 to 2 months before surgery (V1). LMEP and EEG recording were acquired 2 weeks after surgery (V2-LMEPs recording); as well as 1 month (V3), 3 months (V4) and 6 months (V5) after VNS implantation. For the LMEPs recordings, trains of 7s were delivered at increasing current, up to the routine stimulation intensity +0.25 mA allowing to build dose response curves. EEG recordings were performed using a 64-channel cap. For the EEG part, clinical parameters of stimulation (intensity, pulse width, frequency) were kept stable and 180s of EEG with eyes open (EO) and 180s of eyes closed (EC) were recorded. Weighted Phase Lag Index (wPLI – a connectivity metric) was computed in each frequency band (delta, theta, alpha, beta and broadband) during VNS ON and VNS OFF epochs. Using paired t-tests, these values were compared between conditions (VNS OFF/VNS ON) and across visits.

Results

So far, 6 patients were included (3 females, 3 males) and completed V1, V2 and V3; 5 patients finished V4 and 3 patients completed the full study (V5). LMEPs were recorded in patient 1 (V2,V4,V5), patient 3 (V4,V5) and patient 6 (V2,V3,V4) while no LMEPs were recorded in other patients yet (patient 2, 4 and 5). Based on these available data, intensity threshold leading to LMEPs induction showed to decrease over time in one patient (patient 1) (V2-0,375mA; V4/V5-treshold 0,25mA) while no change was observed in the two others (patient 3, 6) (threshold 0,375mA). In 6/6 patients, a significant higher whole brain wPLI was found in V3-V4 compared to V1 during VNS ON in the delta band (p=0,021) and in the theta band (p=0,049) respectively, in EO and EC conditions, while no significant effect was found in the other bands or during the VNS OFF condition. However, a trend can be observed between V1-V3/V4 (p= 0,097) and V4-V5 (p=0,097) in delta band in EC conditions (Fig.1).

Conclusion

LMEPs may depend not only on the intensity of stimulation but also on the recovery of the nerve. VNS may (i) increase wPLI in the initial months after surgery and (ii) decrease wPLI after 6 months in delta and theta bands. These preliminary results may reflect modulatory effects of VNS over time.

Keywords: LMEPs, VNS, EEG

Involvement of the system x_c^- in the context of chronic pain of inflammatory origin

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Background and aims : Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It occupies a central place in public health concerns because even if acute pain is well treated, this is not the case for chronic pain. Traditional analgesics often offer only limited relief for this condition. As a result, one in five individuals experiences chronic pain. It is a frequent reason for medical consultation and is associated with an increased incidence of mental health problems, opiate dependence, and a reduced quality of life.

Regarding the mechanisms responsible for chronic pain, a hypothesis regularly put forward is based on the presence of a disturbance of glutamatergic homeostasis as well as persistent inflammation that are responsible for peripheral and central sensitization. Many evidence lead us to propose that the cystine-glutamate exchanger known as system xc- is involved in the establishment or maintenance of pain sensitization as it influences both glutamatergic signaling and the inflammatory responses.

Methods: In order to evaluate this hypothesis, we worked with a model of chronic pain of inflammatory origin (injection of Complete Freund adjuvant in the hindpaw - CFA) in control mice and transgenic mice with invalidated system xc- (lacking the specific subunit xCT). First, behavioral studies evaluating paw edema, allodynia (Von Frey filaments) and hyperalgesia (thermal paw stimulation) were conducted to appreciate inflammation and pain sensitivity. Then, we studied the expression of xCT as well as different inflammatory and glial markers in the ipsilateral dorsal horn of the spinal cord by RTqPCR. The activity of the exchanger was also evaluated using a validated uptake assay, as well as the level of expression of different inflammatory cytokines in peripheral blood by performing an multiplex antigen detection assay.

Results: Our results comparing the consequence of CFA injection in the paw in xCT+/+ and xCT-/animals show a reduction of edema, mechanical allodynia as well as thermal hyperalgesia in mice lacking functional system xc-.

Besides, the quantification of selected cytokines in the plasma evidenced that xCT+/+ animals injected with CFA exhibit a pro-inflammatory profile, which was not observed in xCT-/-. CFA injection in the different animal genotypes also induces slight differences in the level of mRNA coding for microglial and astrocytic markers.

Conclusion: Based on these experimental observations, we suggest that system xc- may be involved in the establishment and/or the maintenance of inflammatory pain, since its genetic suppression significantly alleviates both peripheral inflammation and pain-related symptoms. This study opens original perspectives for the treatment of patient suffering from chronic pain.

Keywords: Chronic Pain, SLC7A11, inflammation

Modulation of Vagus Nerve Activity in Genetic Absence Epilepsy Rats from Strasbourg (GAERS) During Absence Seizures

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RATIONALE Epilepsy, impacting 50 million people, poses challenges for one-third who are drug-resistant and ineligible for surgery, especially those with childhood absence epilepsy.[1] Vagus nerve stimulation (VNS) serves as an adjunctive treatment, but 30% of patients don't respond effectively. Early vagus nerve stimulation at the seizure stage improves outcomes. Thus, non-invasive seizure detection through autonomic changes visible in vagus nerve neurogram recordings (VENG) could enhance closed-loop stimulation. All seizure types, including absences, exhibit autonomous changes [2,3,4]. Using the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model, mirroring human characteristics [5,6], we aim to detect absences through autonomic changes in VENG and explore the relationship between epilepsy evolution and VENG modulation during seizures.

METHODS The left cervical vagus nerve was implanted with a cuff electrode (outer diameter: 1.6 millimeters, inner diameter: 0.3 millimeters, interelectrode distance: 4 millimeters) for VENG. Epidural electrodes were also implanted for scalp electroencephalography (EEG) monitoring with ground left and right: AP: -2, ML: +-3; Parietal left and right: AP: 5, ML: +-3 and Reference]: AP: -6, ML: 0 under sevoflurane anesthesia. VENG, EEG, and video were recorded 24 hours in 4, 6, and 10-month-old freely moving rats (N=4x3). Ictal VENG segments were identified based on corresponding ictal EEG patterns. Absences preceded by non-rapid eye movement (NREM) sleep were selected for a clean pre-ictal phase of 10 seconds, and seizures lasting less than 5 seconds were discarded. The root mean square (RMS) value of each VENG segment during absences was calculated and compared to the RMS value of the pre-ictal VENG segment. The modulation of the VENG signal compared to the pre-ictal phase is expressed as a ratio. To evaluate the severity of the seizure, the duration, number, and main component frequency of the seizure were analyzed.

RESULTS The vagus nerve activity increases during periods of absence in GAERS. In a sample of 12 GAERS and 674 seizures over the course of 24 hours, the RMS value of vagus nerve activity was found to be greater during seizures than at baseline. Additionally, it was observed that vagus nerve activity during absence in GAERS decreases with increasing age, with a significant difference noted between the 4 and 6-month and 4 and 10-month age groups ($p<0.001$). Furthermore, the variability of the seizure ratio was found to decrease with age, with median values of 1.97±1.46 standard deviations at 4 months, 1.67±0.83 standard deviations at 6 months, and 1.53±0.48 standard deviations at 10 months. The characterization of seizures has been done without showing any difference between age.

CONCLUSION Vagus Nerve Activity chronically recorded on 12 GAERS, show a global RMS value **increase during absences** compared to baseline during NREM sleep. The detection of this modulation could be used as a biomarker for seizure detection and development of closed-loop VNS. Furthermore, the **parasympathetic response** during seizures in GAERS seems to **decrease with age despite the same type of seizure,** which may reflect **autonomic dysfunctions** with the duration of epilepsy.

Keywords: Absence-seizures; Autonomic-system; Epilepstogenesis

Beneficial role of physical activity in counteracting musculoskeletal disorders induced by early movement restriction

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Insufficient physical activity is often associated with sedentary lifestyle. Several organizations have recently highlighted that many children and adolescents are not enough physically active, putting their current and future health at risk. The problem of low physical activity is particularly prevalent in children with chronic illnesses or accident that require prolonged bed rest**.** Similarly, children with developmental coordination disorders (DCD) exhibit sensorimotor integration deficits that result in clumsiness, slowness, and inaccuracy in performing daily motor tasks, as well as cognitive and executive function disorders. Physical hypoactivity and sedentary lifestyle during childhood can lead to various disturbances throughout the sensorimotor pathway such as behavioural, metabolic, spinal or muscular alterations and predispose to numerous future health complications, in particular the emergence of non-communicable diseases. Therefore, understanding the different mechanisms of use-dependent plasticity is crucial for children experiencing early hypoactivity.

Otherwise, nowadays, physical activity is well known to benefit general health, in particular for muscles and brain. Indeed, physical activity is known to improve cerebral plasticity, cognitive and motor functions and seems to be necessary for the harmonious maturation of central nervous system. However, despite the importance of this topic, the ability of physical activity to counteract the deleterious effects of sensorimotor restriction (SMR) during childhood remains to be demonstrated.

In order to mimic the effects of early SMR, a rat model was developed, which consists in immobilising the pups' hindlimb from birth to postnatal day 28 (P28). SMR rats show a major motor impairment characterized by muscle weakness, altered cognitive and executive functions, locomotor disturbances... The aim of the present study was to determine whether physical activity can reverse the muscle alterations induced by early SMR. For this purpose, pups were assigned to Control (CTRL) or SMR groups at birth. From P28 to P60, animals were either housed either in standard cages (sedentary group, SED) or in enriched cages with access to activity wheel for one hour/day (activity group, ACT). At P60, rats were euthanized and three hind limb muscles (slow postural *soleus* – SOL; fast *extensor digitorum longus* – EDL; fast *tibialis anterior* – TA) were removed. Fibre cross-sectional area and myosin heavy chain (MHC) fibre type were determined by immunohistochemistry.

Our results showed a strong atrophy of SOL in the SMR-SED group compared to CTRL-SED group. Physical activity significantly reduced but did not totally prevent atrophy (SMR-ACT group). Interestingly, exercise had no effect on the CTRL group. Fast muscle (EDL and TA) cross-sectional area was not affected by either ACT or SMR. For MHC fibre type, no difference was observed for SOL and TA, whereas a fast-to-slow transition was observed in the EDL for the SMR-SED group.

In conclusion, early SMR from birth to P28 has persistent detrimental effects that are not completely prevented by further exercise from P28 to P60. Nevertheless, physical activity seems to be an interesting countermeasure to reverse some of the changes induced by the early RSM.

Keywords (3): Hypoactivity, Physical activity, Muscle plasticity

Development of activated CAR macrophages for an anti-tumor immunotherapy strategy

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There is increasing interest in immunotherapeutic approaches to cancer. Chimeric antigen receptor (CAR) T-cell therapy has proven effective in the treatment of hematological tumors; however, its efficacy in the treatment of solid tumors is hampered by lower intra-tumor infiltration of CAR T cells and tumor-induced immunosuppression. Macrophages, which constitute a significant part of the tumor environment, play multiple roles in tumor development and represent promising therapeutic targets. Macrophages can infiltrate solid tumor tissue, interact with various cellular components in the tumor microenvironment, and promote a direct anti-tumor response by phagocytosing tumor cells.

In this study, we developed macrophages expressing a CAR receptor against the HER2 antigen. The CAR receptor has an intracellular domain CD3ζ with homology to the protein FcεRI-γ. When activated by the antibody-antigen recognition complex, this domain induces the phagocytic activity of macrophages. Approximately 30% of macrophages express the CAR after transduction. CAR-M exhibited significantly enhanced phagocytosis of HER2-coated beads compared to wild-type (WT) macrophages. CAR-M also demonstrated the ability to phagocytose HER2+ cancer cell lines. Co-culture experiments with breast cancer tumoroids (HER2+ or HER2-) confirmed the efficacy of CAR-M in a more complex environment. However, within the tumor microenvironment, macrophages tend to adopt an anti-inflammatory phenotype, which reduces their anti-tumor activities. To address this issue, we implemented a dual strategy by inhibiting two proprotein convertases, Furin and PC1/3, in CAR-M. Inhibition of furin or PC1/3 resulted in increased pro-inflammatory markers and sustained macrophage activation in the presence of cancer cells. In addition, CAR-M with siFurin or siPC1/3 showed increased phagocytic activity against HER2+ beads or HER2+ tumors. These enzymes proved to be critical phenotypic regulators of macrophages.

Our therapeutic strategy is based on the dual activation of tumor-infiltrating macrophages. The first activation involves enhancing the phagocytic activity of macrophages by expressing a CAR receptor targeting a tumor antigen. The second activation involves reprogramming macrophages towards a pro-inflammatory phenotype by inhibiting Furin or PC1/3 proprotein convertases. The ultimate goal of this therapy is to extend its use to other solid tumors, particularly glioblastoma, an aggressive CNS tumor with limited therapeutic options.

Keywords: Breast Cancer/Glioblastoma, Immunotherapy, CAR-Macrophage

Development of an in vivo ImmuneScore in the case of Glioblastoma via the SpiderMass technology

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Infiltration of various population of immune cells was found to be associated to the patient outcome in colon cancer and is now recognized in more and more cancers. Nowadays, the ImmuneScore is obtained post-surgery from excised tissues or biopsies by immunohistochemistry (IHC) using antibodies specific of the different immune cell populations.

SpiderMass is a technology based on ambient ionization mass spectrometry developed by the laboratory PRISM as a diagnosis or prognosis tool *in vivo* directly in the operating room. Here, we explored the possibility to create an ImmuneScore based on SpiderMass data that could in the future be exploited *in vivo*. To this end, we analyzed with SpiderMass different population of immune cells namely macrophages (M1-like and M2-like), lymphocytes versus NCH82 glioblastoma cells.

A Python library called LGBM was used to train an immune scoring model for which a correct classification rate of 100% was obtained. Using this model, we were able to find immune cells lipids biomarkers like the ions *m/z* 818.65 (GlcCer d18:1_22:0) and 819.55 (PG 18:1_22:6) specific M1-like and M2-like macrophages respectively.

Most interestingly, from a SpiderMass images, we were able to get the predicted distribution of the immune cells across the tissue. Indeed, a pipeline was dedicated to predict the probability of presence of each cell type based on SpiderMass images of 6 FF glioblastoma tissues. The ratios provide insight into the distribution of the trained cell types across the image, allowing a comprehensive assessment of the cellular landscape in patient with shorter versus longer survival.

Moreover, the lymphocytes were predicted to have a higher abundance in tissue from patients with >36-month survival as well as M1-like macrophages. Conversely, M2-like macrophages is highly expressed in patients with shorter survival. Finally, the ratio of M1-like to M2-like macrophages could serve as a prognosis marker.

Our newly developed immunescoring pipeline was confirmed thanks to a 5-plex MALDI-IHC panel. It corroborates our result thanks to the use of antibodies conjugated with novel photocleavable mass-tags. Of particular interest, the SpiderMass-MSI approach, based on immunescoring, allows the differentiation of various subpopulations within the tumor microenvironment without necessitating techniques reliant on probe utilization. In conclusion, this innovative approach not only offers insights into the presence of immune cells within the TME but also presents the potential for a more rapid prognostication of survival time among GBM patients.

Keywords : Mass spectrometry – Artificial intelligence – ImmuneScore

Gastric Biofeedback in Virtual Reality: a Pilot Study

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Gastric Biofeedback (with electrogastrography; EGG) is a promising new tools in the field of interoception, emotion regulation and eating disorders (Davey et al., 2023; Stern et al., 2004; van Dyck & Lutz, 2022; Vujic et al., 2020). However, research in the domain is still scarce with not even a handful of experimental studies on the topic (Stern et al., 2004; Vujic et al., 2020). The current pilot study, therefore, evaluated a novel gastric biofeedback paradigm in virtual reality (VR). We conducted a randomised controlled study with three groups (1) a VR based gastric biofeedback paradigm, (2) the same paradigm in 2D and (3) a relaxation control group. The primary outcome was the extent to which healthy subjects could increase their normal (3cpm) gastric myoelectric activity. Secondary outcomes were motivational aspects and attentional focus. Results showed significant differences between the groups regarding both the increase in 3cpm activity as well as motivational and attentional aspects. This pilot study contributes important new findings in the domain of gastric biofeedback, demonstrating that an increase in normal gastric 3cpm activity through EGG biofeedback is feasible. However, more research is needed to clarify the underlying mechanisms as well as how and if training success may be associated with beneficial effects on interoception, emotional regulation and eating disorder symptoms.

Keywords: Biofeedback, electrogastrogram, virtual reality

Investigating Apex1 as a Key DNA Repair Protein in the Enteric Nervous System

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Damage to mammalian DNA is an inevitable consequence arising from reactions with endogenous chemicals, notably reactive oxygen species, or exposures to environmental genotoxins. To mitigate the harmful effects of DNA damage, organisms have evolved intricate DNA repair mechanisms. The enteric nervous system (ENS), located in close proximity to the gut lumen, lacks the confines of the blood-brain barrier or skeletal structures that protect primary central nervous system components. Consequently, the ENS is susceptible to genotoxic effects, not only from endogenous chemicals but also from circulating or ingested agents such as inflammatory molecules, pathogens, and environmental contaminants. These unique characteristics suggest a heavy reliance of the ENS on DNA repair mechanisms to avert the detrimental consequences of DNA damage. Remarkably, despite its critical role, the significance of DNA repair in the ENS remains largely unexplored. This study endeavors to elucidate the role of a pivotal DNA repair protein, Apex1, as a guardian of the ENS genome. We examined Apex1 expression patterns throughout the aging process in the mouse ENS. Our observations reveal that the population of enteric neurons exhibiting high nuclear Apex1 immunoreactivity remains relatively stable across different life stages. Intriguingly, however, enteric neuron γH2AX expression, as a proxy for DNA damage, significantly increases with advancing age. Additionally, we probed Apex1 expression in distinct enteric neuronal subtypes during adulthood. This analysis unveiled variations in the proportions of subtypes exhibiting high nuclear Apex1 immunoreactivity, with a higher prevalence among nitrergic neurons compared to calbindin-positive enteric neurons. Ongoing experiments are poised to unveil the ENS expression patterns of Apex1 during various developmental stages. Collectively, these findings promise to provide crucial insights into the distribution of Apex1 expression within the ENS. This knowledge will serve as an essential foundation for future investigations aimed at unraveling the developmental and neuroprotective role of Apex1 within the ENS, leveraging various Apex1 deficient mouse models.

Keywords: enteric nervous system, DNA repair, DNA damage

miR-146a-5p Regulation of Enteric Glial Cell Status in Gastrointestinal Health and Disease

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Background: Enteric glia, the glial cell population of the enteric nervous system (ENS), are crucial for maintaining gastrointestinal (GI) homeostasis by supporting enteric neuron function, regulating epithelial barrier integrity, and engaging in communication with the microbiome and immune system. The phenotype of enteric glia is niche-specific and largely determined by micro-environmental cues. In response to injury and intestinal inflammation, enteric glia acquire a reactive phenotype, which may contribute to GI pathophysiology and ENS repair. Previously, *Sox10-Cre^{ERT2}:nuclearGFP* mice infected with Heligmosomoides polygyrus, eliciting an interferon-gamma (IFN-γ)-response signature, intestinal damage, and enteric gliosis were subjected to bulk small RNA sequencing seven days post-infection to identify the enteric glia-miRNA transcriptional landscape of the tunica muscularis at homeostasis and disease (Holland et al., in preparation). This indicated that distinct miRNA signatures can mark enteric glial identity and suggested the potential involvement of miR-146a-5p in establishing the reactive state of enteric glial cells. Nevertheless, further investigation is required to elucidate the precise function of this miRNA in enteric glia in GI health and disease.

Objective: In this study, we aim to explore the role of miR-146a-5p in enteric glial cell reactivity in GI homeostasis and disease.

Methods: To discern the role of miR-146 in enteric glia cell reactivity, we characterized an *in vitro* model of reactive enteric glia induced by treating primary mouse enteric glial cells with lipopolysaccharide (LPS) and IFN-γ for 24 hours. Furthermore, in order to achieve transfection of enteric glia, we embarked on a magnetofection assay. A conditional enteric glia-specific miR-146 knockout model will be used for *in vivo* analyses.

Results: Reactive enteric glia exhibited a significant increase in proliferation and acquired a proinflammatory phenotype *in vitro*. Moreover, the enteric glial processes were less to no longer present, while they typically extend in various directions from the cell body and interact with adjacent enteric glial processes. Importantly, of several microRNAs, only miR-146a-5p exhibited a significant upregulation. The magnetofection assay using a pmaxGFP plasmid demonstrated successful transfection of 20-30% of enteric glia. Ongoing experiments using miR-146 inhibitors and precursors (miR-146a-5p miRCURY LNA miRNA Inhibitor and pre-miR-146a-5p miRNA precursor) will determine whether miR-146a-5p is instrumental for the induction of enteric glia reactivity. *Sox10Cre^{ERT2};R26^{tdT};miR146 $^{flox/flox}$* mice will be used to investigate the role of miR-146a-5p *in vivo.* We will compare ENS structure and function upon miR-146a knockout between control and *Sox10Cre^{ERT2};R26^{tdT};miR146$^{flox/flox}$* mice with dextran sulfate sodium (DSS)-induced colitis using immunofluorescence labeling, qRT-PCR, FACS analysis, and GI functionality assays.

Conclusion: In summary, our current findings include the identification of the reactive phenotype of enteric glia characterized by the upregulation of miR-146a-5p. We have also achieved successful transfection of enteric glia for the first time. Moving forward, we anticipate further insights into the impact of miR-146a-5p expression on enteric glial cell reactivity, as well as its potential implications for GI homeostasis and disease.

Keywords: enteric glial cells, enteric gliosis, miR-146a-5p

MicroRNAs in the Enteric Nervous System: Insights from Dicer Knockout Mice

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The enteric nervous system (ENS) comprises ganglionated neuro-glia networks organised in the myenteric and submucosal plexus located in the gut wall. This intrinsic nervous system orchestrates gastrointestinal functioning, including digestion, motility, and mucosal barrier function. MicroRNAs, which are known for their regulatory roles in various cellular processes, are small, non-coding RNA molecules controlling gene expression through post-transcriptional mechanisms. However, which microRNAs are important for ENS development and function and how they are dysregulated in disease remains to be elucidated. Preliminary data from comprehensive microRNA sequencing analyses of enteric glial cells from the small intestine and colon of adult mice revealed that enteric glia display a regionally-specific microRNA profile, suggesting precise microRNA-mediated regulation of the enteric glia transcriptome. In addition, in models of intestinal helminth infection (Heligmosomoides polygyrus) and intestinal inflammation (DSS colitis) the microRNA landscape of enteric glia changes significantly, indicating that enteric glial microRNAs are implicated in gastrointestinal pathophysiology. To further investigate the function of microRNAs in the ENS, we depleted microRNAs using two transgenic mouse models harbouring a conditional ENS cell-specific knockout of *Dicer*, an RNase III enzyme required for microRNA maturation. *Sox10-Cre:Dicer$^{fl/fl}$* mice, in which Sox10 expressing ENS precursors and their neuro-glia derivatives are Dicer deficient, are non-viable and present with impaired ENS cell composition and myenteric plexus structure when examined at embryonic day 17.5. Currently, we are comparing ENS architecture and constituent cell numbers after enteric glia-specific depletion of Dicer in adult *Sox10-Cre^{ERT2}:Dicer$^{fl/fl}$* mice at steady state and after a Heligmosomoides polygyrus infection. These analyses will be complemented with in vivo assessments of gastrointestinal functioning by measuring whole gut transit time and colonic propulsionand intestinal permeability. So far, our results indicate that during embryonic stages, mature microRNAs expressed by ENS progenitors are essential for the normal development of intrinsic intestinal neural networks. By combining structure-composition analyses and gut function experiments, we aim to extend these findings to the role of enteric glia microRNAs in the adult gastrointestinal tract.

Keywords: enteric glia, microRNAs, enteric nervous system

Linking interoception and introspection via insula targeted neurofeedback – A feasibility study

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Interoception is known as the processing and central representation of afferent internal bodily signals. When engaging in interoceptive processes, for example counting one's own heartbeat, the insula region is activated. Alterations in interoception have been linked to alterations in emotion regulation and psychopathologies related to this.

Through real-time functional MRI (rtfMRI) neurofeedback training (NFT) one can try and learn the voluntary regulation of a selected area of the brain. Some NFT studies have assessed participants' ability to estimate their current level of activation of the target region, suggesting that people might have introspection into their neural activity. The aim of the current study was to assess (1) the influence of NFT targeting the insula on interoceptive processes and (2) if people have introspection into their performance of insula upregulation. First, the current study assessed the feasibility of a heart beat counting task to localize the insula. Second, using an intermittent graded neurofeedback design targeting the insula, we assessed the ability to estimate the level of one's own insula activity in the absence of feedback (introspection).

Ten healthy volunteers received three neurofeedback sessions. Each session started with a heart beat counting (interoception) task. Voxels within the insula passing the threshold for the contrast counting (12 trials) > rest were selected as the target region for the subsequent neurofeedback task. Next, three runs (of 8 trials each) of neurofeedback were performed. Within each trial, participants were first asked to upregulate the insula to a certain level (0.6 or 0.9% percent signal change compared to baseline). Second, participants were asked to estimate how active the target region was during the regulation period, and how confident they were about this estimation. Lastly, participants received visual feedback on how active the target region actually was during the regulation period.

Preliminary results show good feasibility of the functional localizer procedure in order to activate the insula. Further analysis will be performed to assess the feasibility of the current paradigm and to develop follow-up studies.

Keywords: fMRI neurofeedback, interoception, insula

The neuropsychological assessment in Dutch memory clinics: a longitudinal perspective

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Background. To improve timely diagnosis of cognitive disorders and dementia the involvement of memory clinics (MCs) in the diagnostic process has been promoted and recommended by national dementia strategies. In the Netherlands, the number of MCs increased from 63 in 2009 to 91 in 2016. Neuropsychological assessments (NPAs) are a cornerstone in the diagnostic process of cognitive disorders in MCs. However, NPA procedures and approaches vary between MCs. The aim of this study is to determine NPA characteristics, such as dedicated time, used cognitive tests, and logistics within MCs in the Netherlands in 2023. Additionally, the results will be compared with earlier findings from 2016 to investigate potential changes in working methods within Dutch MCs.

Method. The NPA monitor, an online questionnaire, has been sent to 73 (neuro)psychologists associated with MCs in the Netherlands. The questionnaire consists of 56 questions on the following topics: qualification of psychologists, procedures/infrastructure, NPA tests and questionnaires, normative data, post-diagnostic case and counselling, and differences in procedures between people with young (< 65 years) versus late onset of complaints. The NPA monitor was sent out in May 2023. After four written and oral reminders, 69 psychologists participated, which means a final response rate of 95%.

Results. Preliminary results showed that NPAs were, on average, conducted 17 times per month per MC, but with a large amount of variation in the duration of the total NPA. Compared to 2016, all MCs still used cognitive screening tests in 2023. The use of the MoCA has increased whereas the FAB, CAMCOG, and MMSE have decreased compared to 2016. 88% used a standardized neuropsychological battery, which all included the cognitive domains memory, attention, executive functioning, and language. Intelligence, perception, praxis, social cognition, and validity tests were less commonly used.

Discussion. At the EURON PhD days, we will present the final results of the 2023 survey and compare these to practices in 2016. The knowledge gained from this survey provides greater insight into the characteristics and procedures of the NPA in Dutch MC settings.

Keywords: Neuropsychological Assessment, Memory Clinics, Questionnaire

Rasch-built Overall Disability Scale for IgM-associated polyneuropathy with and without anti-MAG antibodies (IgM-RODS)

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The IMAGiNe consortium presents the results of the first study aim: the construction of an IgM peripheral neuropathy (IgM PNP)-specific patient-reported outcome measure at the level of activity and participation. IgM monoclonal gammopathy-associated polyneuropathy with or without anti-Myelin Associated Glycoprotein (± anti-MAG) is a rare immune-mediated disease that may cause severe limitations in daily activities and quality of life. The absence of a systematic comparison between patients with anti-MAG positive and negative IgM-polyneuropathy and the lack of international consensus on how to assess and treat them are factors currently obstructing future clinical advances. Furthermore, the clinical trials conducted for this condition have been negative and capturing clinical meaningful changes has been difficult. To guarantee an accurate assessment of functional status, a functional disease-specific outcome measure must be constructed.Therefore, an international observational prospective cohort study was initiated; the IMAGiNe (IgM ± Anti-Myelin Associated Glycoprotein [MAG] peripheral Neuropathy) study.

The primary objective is to develop an interval Rasch-built activity/participation scale specifically for IgM- polyneuropathy ± anti-MAG (IgM-RODS) and examine its clinimetric properties. A pre-phase IgM-RODS questionnaire containing 146 activity/participation items, based on the WHO international classification of Functioning, Disability and Health, was completed by participants of the IMAGiNe study observational registry that fulfilled international criteria for with IgM- polyneuropathy with and without anti-MAG, older than 18 years, and without concomitant diseases affecting nerve function. The data was subjected to Rasch analyses and reliability and validity studies were performed on the final IgM-RODS.

The pre-RODS data of 259 subjects (Denmark: 31, France: 21, Italy 19; Netherlands: 103, Serbia: 8, Spain: 24, UK: 24, USA: 29) underwent quality assessment and 244 remaining records were submitted to the Rasch model evidencing unmet model's expectations. Based on requirements like exceeding fit residual range, misfit statistics, differential item functioning, local dependency, and less face validity we systematically removed items until a final 40-item IgM-RODS was constructed fulfilling all Rasch requirements. Good reliability, construct and discriminant validity values were obtained (PSI= 0.96, R2: 0.95). Compared to the Inflammatory-RODS, the IgM-RODS showed lower standard errors across the metric, indicating greater sensitivity. Furthermore, the diversity of the cohort allowed for a cautious assessment of cultural bias and the achievement of adequate cross- cultural validity.

The IgM-RODS is a disease-specific interval measure suitable for parametric analysis and capable of detecting functional deficit in patients with IgM-polyneuropathy ± anti-MAG. Future studies will be performed to determine the responsiveness of the IgM-RODS. Ultimately, its development helps the research community overcome previous barriers associated with the use of inconsistent suboptimal ordinal measurement tools.

Keywords: Clinimetry, IgM-polyneuropathy, Rasch-built

Association between visual short-term memory binding performance and tau burden in the medial temporal lobe

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Background: Alzheimer's disease (AD) starts with a preclinical stage during which amyloid (Aβ) and tau pathologies develop in the brain while patients remain cognitively normal (CN). The correspondence between the regional distribution of these proteinopathies in the brain and cognitive impairment better matches the established brain-behavior relationships for tau than for the Aβ pathology. The medial temporal lobe (MTL) is the first regions to demonstrate abnormal tau accumulation in AD. The MTL is implicated in discriminating very similar objects by implementing the binding between their multiple features. The Visual Short-Term Memory Binding Test (VSTMBT, Parra et al., 2010) was showed to solicit the MTL. However, impaired performance on this task since the preclinical stage of sporadic AD in relation to tau burden in the MTL is not clearly established.

Methods: 68 older adults underwent a [F18]-MK6240 tau-Positron Emission Tomography (PET) to quantify tau burden in the MTL (entorhinal cortex, hippocampus, parahippocampus), a Magnetic Resonance Imagery (MRI), either lumbar punction or [F18]-Flutemetamol PET to determine the Aβ status, a standard cognitive assessment and the VSTMBT. In VSTMBT, the participant had to determine whether a set of shapes were identical to the set presented before a blank retention interval of 900ms. There were 4 conditions in which the number of shapes (2 or 3) and the color (white= “Shape-Only condition” or colored) of the shapes varied. The colored conditions implied to memorize the binding between shapes and their respective color (“Shape-Color Binding”). We calculated the accuracy score (AS) and the mean reaction time (RT) for each condition. Participants were classified in three diagnostic groups based on the Aβ status and the standard neuropsychological assessment: Aβ-CN (n=35); Aβ+ CN (or preclinical AD, n=16); and Aβ+ mildly cognitively impaired participants (Aβ+MCI, n=17).

Results: The AS was lower in Aβ+CN than in Aβ-CN participants in all conditions except the

Shape-Only-2-items, while Aβ+CN and Aβ+MCI did not differ. The Aβ+CN group was slower than Aβ-CN only in the Shape-Color Binding-2-items. There was no interaction between diagnostic group and condition, nor between group and the number of items.

There was an interaction between condition and MTL tau burden: the MTL tau burden was more strongly associated with performance in the Shape-Color Binding conditions than in the Shape-Only conditions, even when adjusting for age and education. A similar effect of MTL tau on binding performance was observed in CN only (n=51), but not within the Aβ+CN, possibly following lack of power.

Discussion: Impaired visual short-term memory performance was evidenced since the preclinical stage of AD, including in binding conditions. Performance in binding conditions was related to tau burden in the MTL, suggesting that impairment in visual short-term memory binding abilities may constitute an early cognitive marker of accumulating tau pathology in the MTL.

Keywords: Alzheimer’s disease, diagnostic, cognition

The association between cerebrospinal fluid AT biomarker profiles and the different *APOE* genotypes across age in persons without dementia

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Background

Amyloid-β deposition (A) and tau tangle formation (T) are the key mechanisms behind Alzheimer's disease (AD). A genetic component considered to be a risk factor in the development of AD is the

Apolipoprotein E (*APOE*) ε4 allele, while the *APOE* ε2 allele is considered to be a protective factor. Promoting a fruitful strategy to either prevent or delay AD progression in those with different *APOE* genotypes requires a better understanding of the frequency of AT biomarker profiles across *APOE* genotypes and age in the various pre-dementia stages.

Methods

A total of 8353 participants were selected from 45 studies that participated in the Amyloid Biomarker Study based on availability of data on age, CSF amyloid-β_{42} (A), CSF phosphorylated tau (T), and *APOE* genotype (ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε3ε4, ε4ε4). The participants were classified as having normal cognition (NC; *N* = 4356) or mild cognitive impairment (MCI; *N* = 3997). Based on normal or abnormal values for A (data-driven) and T (center-specific cutoffs) participants were placed into 4 AT biomarker profiles (A-T-, A+T-, A-T+, A+T+). For each diagnostic group (NC, MCI) a Markov-Chain-Monte-Carlo generalized linear mixed model was performed to evaluate the association *APOE* genotype and age with the AT profiles.

Results

The NC group had an average age of 65.10 years (*SD* = 10.78), with 59% being female. The MCI group had an average age of 69.30 years (*SD* = 8.44), with 48% being female. Most participants with had the *APOE* ε3ε3 genotype (NC 56% and MCI 45%). For an overview of the demographic results, see Table

1. In both NC and MCI, the association of AT profiles with *APOE* genotype changed across age ($p <$

.001; Figure 1). In participants with NC and the *APOE* ε2ε2 genotype, nearly 100% had A-T- across

age. For those with *APOE* ε2ε3 and ε3ε3 genotypes, A-T+ was relatively frequent in older age compared to the other AT profiles. In those with at least one ε4 allele A+T- became relatively frequent at older age. However, in the *APOE* ε3ε4 and ε4ε4 genotypes A+T- frequency seemed to plateau around 65 years of age. Around the same age A+T+ strongly increased in frequency. For those with MCI, A+T+ was most frequent with aging across all *APOE* genotypes.

Conclusion

Frequencies of AT biomarker profiles in persons with NC or MCI changed substantially across *APOE* genotypes and age. This may be important in the prescreening of AT profiles for trials in those without dementia.

Table 1.

Demographic variables

	Normal cognition					Mild cognitive impairment				
	Total Sample (*N* = 4356)	**A-T- (*N* = 2471)**	**A+T- (*N* = 1094)**	**A-T+ (*N* = 439)**	**A+T+ (*N* = 352)**	**Total Sample (*N* = 3997)**	**A-T- (*N* = 1180)**	**A+T- (*N* = 815)**	**A-T+ (*N* = 533)**	**A+T+ (*N* = 1469)**
Age										
Mean (SD)	65.10 (10.78)	63.11 (11.16)	65.76 (10.20)	69.70 (8.59)	71.31 (7.43)	69.30 (8.44)	66.01 (8.77)	69.25 (8.35)	70.52 (8.11)	71.52 (7.44)
Sex										
Female (%)	2566 (59%)	1463 (59%)	644 (59%)	270 (62%)	189 (54%)	1935 (48%)	560 (47%)	338 (41%)	261 (49%)	776 (53%)
Male (%)	1790 (41%)	1008 (41%)	450 (41%)	169 (38%)	163 (46%)	2062 (52%)	620 (53%)	477 (59%)	272 (51%)	693 (47%)
Education										
Mean (SD)	14.41 (3.83)	14.28 (3.72)	14.70 (4.05)	14.31 (3.89)	14.49 (3.76)	12.22 (4.35)	12.26 (4.11)	12.40 (4.44)	11.78 (4.60)	12.23 (4.40)
MMSE										
Mean (SD)	28.91 (1.93)	28.97 (1.33)	28.82 (1.55)	28.94 (1.16)	28.62 (1.69)	26.89 (2.56)	27.60 (2.18)	26.94 (2.65)	27.06 (2.37)	26.22 (2.68)
APOE genotype										
ε2ε2 (%)	16 (0.4%)	15 (0.6%)	1 (0.1%)	0 (0.0%)	0 (0.0%)	9 (0.2%)	6 (0.5%)	1 (0.1%)	1 (0.2%)	1 (0.1%)
ε2ε3 (%)	439 (10.1%)	292 (11.8%)	84 (7.7%)	51 (11.6%)	12 (3.4%)	270 (6.8%)	143 (12.1%)	44 (5.4%)	41 (7.7%)	42 (2.9%)
ε2ε4 (%)	102 (2.3%)	54 (2.2%)	31 (2.8%)	9 (2.1%)	8 (2.3%)	83 (2.1%)	26 (2.2%)	22 (2.7%)	9 (1.7%)	26 (1.8%)
ε3ε3 (%)	2433 (55.9%)	1527 (61.8%)	512 (46.8%)	265 (60.4%)	129 (36.6%)	1812 (45.3%)	763 (64.7%)	336 (41.2%)	300 (56.3%)	413 (28.1%)
ε3ε4 (%)	1221 (28.0%)	552 (22.3%)	405 (37.0%)	104 (23.7%)	160 (45.5%)	1394 (34.9%)	226 (19.2%)	291 (35.7%)	169 (31.7%)	708 (48.2%)
ε4ε4 (%)	145 (3.3%)	31 (1.3%)	61 (5.6%)	10 (2.3%)	43 (12.2%)	429 (10.7%)	16 (1.4%)	121 (14.8%)	13 (2.4%)	279 (19.0%)

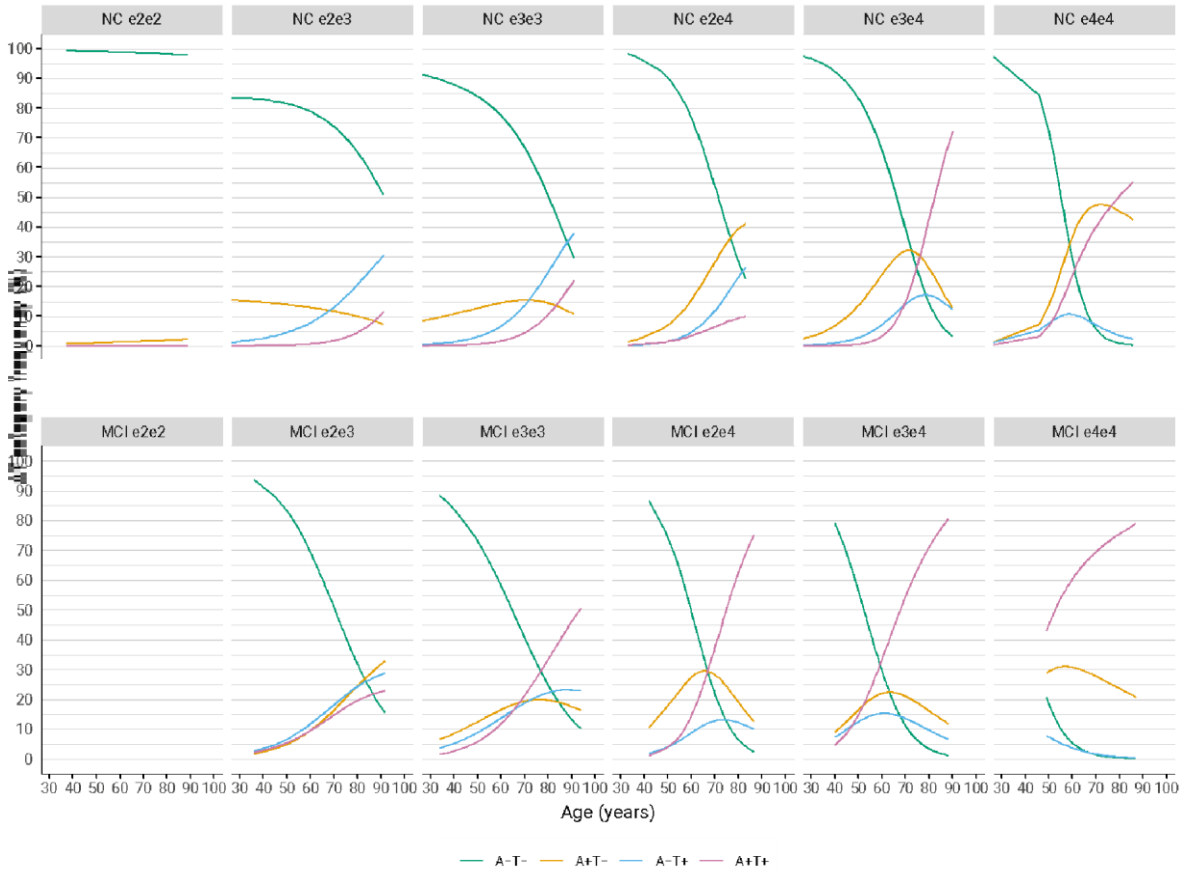

Figure 1. Frequencies of AT biomarker profiles across *APOE* genotypes and age for persons with normal cognition (NC) and mild cognitive impairment (MCI).

Keywords: *APOE* genotypes, AT profiles, Alzheimer's disease

Study of *RFC1* physiopathology in parkinsonism

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Background. Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a neurodegenerative disease with a genetic cause discovered in 2019 by Cortese et al. They described a mutation in intron 2 of *RFC1* gene consisting of a repeat expansion with a modification of the expanded motif. The $(AAAAG)_n$ or $(AAAGG)_n$ motifs are replaced by a pathogenic $(AAGGG)_n$ motif on both alleles of the gene. Several recent studies report cases of patients carrying a pathogenic expansion on one allele and one nonsense, frameshift or splice mutation on the other. These patients also exhibited a CANVAS phenotype associated with a decrease of *RFC1* mRNA level in the peripheral whole blood or in the fibroblast. These studies tend to support that the impact of pathogenic *RFC1* mutations relies on a loss-of-function mechanisms. Rare cases of *RFC1* biallelic expansions have been reported in cohorts of patients with different parkinsonian syndromes like multiple system atrophy and Parkinson's disease. Moreover, our team revealed that 10% of CANVAS patients had parkinsonism, a rate 10-fold higher than what is expected in a matched healthy population of similar age. We also reported the neuropathological examination of a patient with advanced CANVAS, parkinsonism and dementia that showed the presence of Lewy bodies in the *locus coeruleus* and *substantia nigra* as well as neuronal loss in the *substantia nigra*. Our hypothesis is that $(AAGGG)_n$ expansions in the *RFC1* gene induces a loss-of-function, presumably responsible for parkinsonism.

The aim of this PhD is to provide an extensive characterization of the role of *RCF1* in parkinsonism.

Methods. Firstly, we will screen for *RFC1* pathogenic expansions in four cohorts of patients with different parkinsonian syndromes (sporadic, inherited or atypical Parkinson's disease and multiple system atrophy cohorts) and in the Lille brainbank to study the association between *RFC1* and parkinsonism. Secondly, to address the pathological effect of a loss-of-function of *RFC1*, we will induce a knock-down of the gene using siRNAs in LUHMES cells and N1E115 cells. Lastly, we will take profit of this *in cellulo* study to perform a knock-down of *Rfc1* in mouse using striatal injections of an AAV-vector enabling retrograde transduction and allowing the expression of three shRNAs targeting *Rfc1* in the *substantia nigra*. We will performed behavioral testing before and after injection (Open field, himb-limb clasping, inverted grid test, rotarod, challenging beam, pole test, Y-maze, novel object recognition test and elevated plus maze) and neuropathological analyses to look for dopaminergic neurons loss or dysfunction in the *substantia nigra*.

Results. So far, we uncovered 7/2261 (0.3%) of biallelic $(AAGGG)_n$ *RFC1* expansions in our four cohorts. These patients' phenotypes consist in five atypical Parkinson's disease and two probable multiple system atrophy.

Discussion. Together, these observations and our preliminary data favour an association between *RFC1* mutations and parkinsonism. However, the mechanisms leading to neurodegeneration in *RFC1* patients are not understood. This is why we are continuing our study with the knock-down of *RFC1 in cellulo* and *in vivo*, to address the loss-of-function of this gene in parkinsonism.

Keywords: Repeat expansions, parkinsonism, *RFC1*.

Insights from the 'Memory Clinic Monitor 2023'

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Introduction: Between 1998 and 2016 the number of memory clinics (MC) in The Netherlands increased from 12 to 96. Diagnostics for cognitive disorders have been improved tremendously by means of these memory clinics. The 'Memory Clinic Monitor' is a questionnaire that has been distributed several times (1998, 2004, 2009 and 2016) among Dutch MC's. The aim of this study is to investigate the organization and methods of the current Dutch memory clinics and to investigate changes over the years since 1998.

Methods: The 'Memory Clinic Monitor 2023' is an online questionnaire that was distributed in July of 2023 among clinicians of the 91 MC's in The Netherlands. The questionnaire consists of 88 questions about organization, collaboration, patient characteristics, procedures, scales and questionnaires, neuropsychological assessment, additional investigations, novel biomarkers, disclosure of the results and diagnosis, post-diagnostic care, treatment and support, changes because of the COVID-19 pandemic and some general questions. Preliminary findings are based on 47 MC's. Complete results are expected around January 2024.

Preliminary results: Preliminary results of the Memory Clinic Monitor 2023 showed a further increase in number of patients referred to an average of 320 per MC, from the 268 average in 2016. MC's are most commonly embedded in the clinical geriatrics department (66%), followed by neurology (60%) and elderly care (13%). The most common medical disciplines working at the MC are neurologists (38 MC's) and clinical geriatricians (34 MC's). Psychologists were present at 42 MC's. The current preliminary results showed a decrease to 47% of patients being diagnosed with dementia. This is a further decrease from the 85% in 1998 and the 53% in 2016. The portion of patients who received a diagnosis of less severe cognitive disorders such as Mild Cognitive Impairment (MCI) increased from 10% in 1998 to 25% in 2016, in the Memory Clinic Monitor 2023 this showed to further increase to 29%.

Conclusion: The results of the 'Memory Clinic Monitor 2023' will be presented at the Euron PhD days. The focus will be on the developments of the memory clinics since 2016. This study will provide a good overview of the current methods and organization of memory clinics in The Netherlands.

Keywords: memory clinic, dementia, diagnostics.

The atrophy of specific amygdala subnuclei is associated with mesiotemporal tauopathy in preclinical AD individuals

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Background: Alzheimer's disease (AD) is characterized by the accumulation of amyloid-beta deposits and tau tangles, starting in cognitively normal (CN) adults, and leading to progressive cognitive impairment and dementia. In AD, tauopathy starts in the mesio-temporal lobe, including the amygdalahippocampal complex. These anatomical structures are respectively composed of subnuclei and subfields that until recently could not be distinguished in-vivo. This possibility was recently implemented in FreeSurfer 7. We aimed to investigate the relative atrophy of the hippocampus and amygdala subregions in preclinical AD, specifically to evaluate whether the atrophy of specific subregions could inform about early tauopathy before cognitive impairment is noticeable.

Methods: We first conducted an exploratory study in the Alzheimer's Disease Neuroimaging Initiative 3 (ADNI3) cohort including 144 amyloid-positive (A+) CN adults for which 3DT1-MRI and [^{18}F]AV1451 Tau-PET data were available. MRI data were processed in FreeSurfer 7. We identified hippocampal and amygdala sub-regions whose volume explained at least 1% of the variance in temporal tauopathy (meta-ROI). These regions were pooled to form two aggregates of tau-associated hippocampal (HA) and amygdala (AA) sub-structures.

We subsequently validated these results in an independent cohort of 112 non-demented subjects from UCLouvain in which 3DT1-MRI and [^{18}F]MK-6240 Tau-PET were available. We evaluated the volume differences in previously defined sub-structure aggregates and in global structures. Individuals were either grouped based on their visual Braak-stage on Tau-PET or on a bioclinical classification (cognition (CN/MCI), amyloid (A-/A+) and tau (T-/T+)).

Results: We first observed in the ADNI3 cohort that neither the global amygdala, nor hippocampal volume were associated with temporal tauopathy in the A+CN group (R^2<0.001). We then identified that only three hippocampal subfields (left hippocampal-tail, body of the left molecular-layer, and body of left subiculum) and six amygdala subnuclei (right and left central and medial nuclei, right accessorybasal and cortical nuclei) each explained more than 1% of the variance in temporal tau. When aggregating these substructures (HA and AA), we observed that they were significantly associated with temporal tauopathy in A+CN individuals, even after adjusting for the global hippocampus and amygdala volumes. In contrast, in the A+MCI (n=132) group, the global structure volumes were significantly associated with temporal tauopathy, and HA or AA did not add additional explanatory power.

In the UCLouvain cohort, we observed that individuals with early tau PET signal (Braak I-II) had significantly smaller volume of the AA than Braak 0 individuals. Similarly, the AA was significantly smaller in A+T+CN compared to A-T-CN. HA, as well as the global hippocampal and amygdala volumes were only reduced in Braak >III or in A+T+MCI subjects.

Conclusion: We identified a set of amygdala subnuclei whose atrophy is earlier than the atrophy of the global amygdala or hippocampus. The atrophy of these specific amygdala subnuclei is associated with temporal tauopathy in preclinical AD individuals, it distinguishes A+T+CN subjects from A-T-CN subjects, and visual Braak I-II from Braak 0 subjects. Measuring amygdala subnuclei volumes in older adults is thus a promising approach to identify individuals at-risk of progression to clinical AD.

Keywords: Alzheimer's Disease, Neurodegeneration, Tauopathy