UNIVERSITY OF MILAN

POSTGRADUATE SCHOOL IN PHARMACOLOGICAL SCIENCES

Department of Medical Biotechnology and Translational Medicine (BIOMETRA)

PhD course in pharmacological science (XXVII cycle)

Synapse and dendrite deficits induced by mutations in the X-linked intellectual disability gene *Il1rapl1*

Sector of study/Settore scientifico-disciplinare
BIO/14

Tutor
Dr. Carlo Sala

Coordinator
Prof. Alberto Panerai

Doctorial Thesis of
Caterina Montani
Registration number/Matricola
R09660

Academic year 2013/2014

Alla Nonna Angela,

e a noi che le vogliamo un gran bene

INDEX

INTRODUCTION

NEURONAL DENDRITES

The brain contains a vast network of almost 100 billion of neurons that are cells specialized in intracellular communication (Purves, 2008). Although the morphology of various types of neurons differs in some aspects, they all contain four distinct regions with differing functions: the cell body, the dendrites, the axon, and the axon terminals (Lodish, 2000).

Most neurons have multiple dendrites, which extend out-ward from the cell body and are specialized to receive chemical signals from the axon termini of other neurons. Dendrites convert these signals into small electric impulses and transmit them inward, in the direction of the cell body. Neuronal cell bodies can also form synapses and thus receive signals. Particularly in the central nervous system, neurons have extremely long dendrites with complex branches. This allows them to form synapses and receive signals from a large number of other neurons (Lodish, 2000).

Dendrites differ from axons in many important aspects, both morphologically and functionally (Craig and Banker, 1994). Dendrites have specialized structures including spines, which are the main excitatory synaptic sites that are not found in axons. Unlike axons, dendrites have tapering processes such that distal branches have smaller diameters than proximal ones. Furthermore, dendrites and axons contain different types of organelles, such as Golgi outposts, found primarily in dendrites. The orientation of microtubules also differs considerably in dendrites and axons; in both vertebrates and invertebrates, the microtubules uniformly orient with their plus-end distally in axons, whereas dendrites contain microtubules of both orientations (Baas et al., 1988; Rolls et al., 2007). It is likely that these different cytoskeletal arrangements influence the manner in which organelles and molecules are transported along axons and dendrites. Given their many structural and functional differences, axon and dendrite development must differ in crucial ways (Goldberg, 2004). Indeed, molecules that function specifically in dendrite or axon growth have been discovered.

Dendritic arbor development is a complex, multi-step process (Fig. a), which generally can be divided, into several different, although partially overlapping, stages: (I) neurite initiation, outgrowth and guidance; (II) branching and synapse formation, and (III) stabilization (Scott and Luo, 2001; Portera-Cailliau et al., 2003; Williams and Truman, 2004).

Although the time scale of these steps differs between species, the sequence of events seems to be very similar. Initial dendrite growth is relatively slow, and a very fast period of dendritic extension follows. For example, total dendritic length increased from ~50 to 100 μm during first 24 h of X. laevis tectal neurons dendritic arbor development but almost 4-fold increase was observed during

the next 48 hours (Wu et al., 1999). Subsequently, dynamic dendritic branching occurs, which combined with neuronal activity and synapse formation, leads to the establishment of a well-developed dendritic arbor. Stabilization of the dendritic arbor occurs over a long period of time (Wu et al., 1999; Williams and Truman, 2004). While the development of dendritic tree associates with high rates of branch additions and retractions, the mature dendritic arbor is less plastic with a very low branch turnover under basal conditions (Wu et al., 1999). Nevertheless, dendritic arbors in the mature nervous system preserve some degree of plasticity (Urbanska et al., 2008).

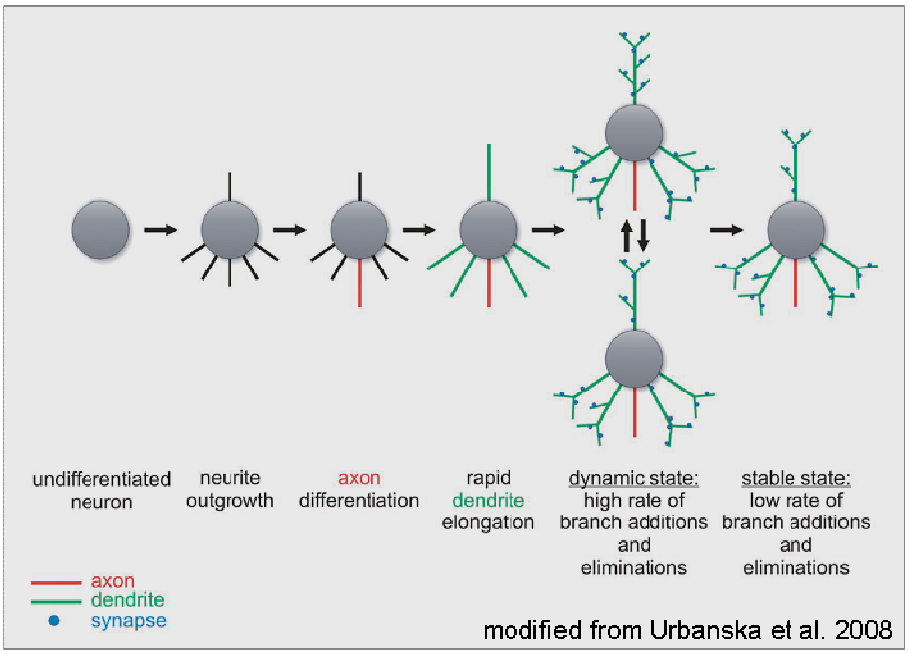


Fig. a. Development of dendritic arbor consists of several overlapping stages (Urbanska et al., 2008).

The complex processes of dendritic arbor development and stabilization must be highly orchestrated at the molecular level. Recent advances in genetic manipulations of neuronal cells helped to reveal a complicated interacting network of dozens, if not hundreds, of proteins involved in signal transduction, macromolecule synthesis, cytoskeleton rearrangements and intracellular trafficking of proteins and membranes. These processes are regulated by both an intrinsic genetic program and a wide variety of extracellular signals, either globally at the whole-cell level or locally in dendrites (Urbanska et al., 2008).

Starting with the seminal work of Ramon y Cayal, the complexity and diversity of dendritic arbors has been recognized and well documented. Transcription factors, receptor–ligand interactions, various signalling pathways, local translational machinery, cytoskeletal elements, Golgi outposts

and endosomes have been identified as contributors to the organization of dendrites of individual neurons and the placement of these dendrites in the neuronal circuitry.

Dendrites must satisfy the following physiological requirements to ensure proper neuronal function. First, a neuron's dendrites need to cover the area (its dendritic field) that encompasses its sensory and/or synaptic inputs (Wassle and Boycott, 1991; MacNeil and Masland, 1998). Second, the branching pattern and density of dendrites must be suitable for sampling and processing the signals that converge onto the dendritic field (Losonczy et al., 2008; Spruston, 2008). Third, dendrites need to have the flexibility for adjustment in development and in response to experience (Jan and Jan).

Many transcription factors contribute to the specification of neuronal type-specific dendrite patterns. A distinct dendrite morphology can be achieved by varying the levels of a single transcription factor, the specific expression of a transcription factor in a single type of neuron and combinatorial mechanisms that involve many transcription factors (Jan and Jan). For instance, the basic helix–loop–helix (bHLH) transcription factor Neurogenin 2 has a crucial role in the specification of dendrite morphology of pyramidal neurons in the neocortex: it promotes the outgrowth of a polarized leading process during the initiation of radial migration (Hand et al., 2005).

Moreover, developing dendrites of invertebrate and vertebrate neurons are responsive to extrinsic signals that can not only stimulate or inhibit outgrowth but can also act as cues for directional growth. For exemple, extrinsic factors such as neurotrophin 3, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) influence the dendrite morphology of cortical neurons (McAllister, 2002).

Regarding the cell biological basis of dendrite morphogenesis, actin and microtubules are the major structural components that underlie dendrite morphology. Regulators of actin and microtubule dynamics therefore have important roles in dendrite morphogenesis. For instance, the Rho family of GTPases control a wide range of cytoskeletal rearrangements that affect spine morphogenesis and the growth and branching of dendrites in both vertebrate and invertebrate neurons (Leemhuis et al., 2004; Newey et al., 2005; Chen and Firestein, 2007; Sfakianos et al., 2007). In addition, motors that mediate the transport of building blocks and organelles to dendrites are crucial for dendrite morphology (Satoh et al., 2008; Zheng et al., 2008). Dendrite growth and branching also require signalling molecules, building materials and organelles such as Golgi outposts and endosomes, which are transported by molecular motors along microtubules (Jan and Jan).

At least three mechanisms contribute to the organization of dendritic fields: self-avoidance, tiling and coexistence. Dendrites of the same neuron avoid one another (self-avoidance) (Jan and Jan).

Dendrites of certain types of neurons —such as class III and class IV dendritic arborization neurons in D. melanogaster — avoid one another (tiling). Self-avoidance and tiling presumably ensure efficient and unambiguous coverage of the receptive field. Furthermore, dendrites of different neuronal types can cover the same region (coexistence) (Grueber et al., 2003) allowing the sampling of different types of information from the same receptive field. These three principles for organizing dendrites rely on different molecular mechanisms, for example, the immense diversity of D. melanogaster Down syndrome cell adhesion molecule (DSCAM) splice variants and the stochastic expression of a small subset of such isoforms in each dendritic arborization neuron ensure self-avoidance without compromising coexistence. Mammalian DSCAM also mediates self-avoidance, indicating conservation of the function of this protein across species (Fuerst et al., 2008).

Furthermore, the size of dendritic arbors increases in proportion to animal growth, a phenomenon known as dendritic scaling. The final size of a dendritic arbor, once reached, is maintained throughout the lifetime of the neuron (Jan and Jan). Whereas some neurons such as the mammalian cerebellar granule cells do not alter their dendrites extensively during later stages of development, the dendrite size and complexity of other neurons such as Purkinje neurons grow in proportion to the animal's growth (Jan and Jan). In addition, different types of neurons may scale their dendrites in different ways depending on their functional requirements. Once neuronal dendrites are sufficiently large to cover the dendritic field of the full-grown animal, it is important that dendritic coverage is maintained (Parrish et al., 2007). The molecular control of dendritic growth, scaling and maintenance is only beginning to be understood. Recently, kinase cascades (Dijkhuizen and Ghosh, 2005; Jaworski et al., 2005; Jossin and Goffinet, 2007; Lee and Stevens, 2007; Parrish et al., 2009) and local translation in dendrites (Ye et al., 2004; Vessey et al., 2008) have been shown to be important for the control of dendrite size of certain mammalian neurons as well as D. melanogaster neurons (Jan and Jan). For instance, the PI3K–mToR kinase pathway, which is well known for its role in controlling cell size, has been shown to regulate dendrite size (Jaworski et al., 2005; Kumar et al., 2005). In addition, this pathway acts together with the mitogenactivated protein kinase (MAPK) cascade to regulate dendrite complexity and branching pattern (Kumar et al., 2005).

Little is known about the mechanisms that are responsible for dendrite maintenance. Extended time-lapse analyses of mouse cortical neurons have revealed that, following a period of juvenile plasticity during which neurons establish normal dendritic fields, dendrites become stable with most neurons maintaining their dendritic fields for the remainder of their lifespan (Grutzendler et al., 2002; Holtmaat and Svoboda, 2009).

The vast majority of stable dendritic spines and filopodia-like protrusions on mature dendrites have synapses (Harris, 1999; Arellano et al., 2007). In vivo studies indicate that synaptic activity promotes dendritic arbor elaboration at early stages of brain development. At later stages of development, synaptic activity stabilizes dendritic structure. The different roles of synaptic activity with respect to structural plasticity probably reflect the regulated spatiotemporal expression of key components within signaling pathways (Cline, 2001).

There are several sources and patterns of neuronal activity that drive brain development, which change as the neurons and circuits within the developing brain become established. One type of activity comes from waves of action potential activity in the retina, hippocampus, cortex and spinal cord, which are present during brain development (Feller, 1999).Waves of depolarization and associated calcium transients can provide activity-based signals both to the neurons participating in the wave directly and to the postsynaptic partners of those neurons. A second type of spontaneous activity, meaning activity that is not driven by afferent inputs, has been recorded in clusters or 'domains' of neurons within limited periods of development (Yuste et al., 1995). Importantly, this type of activity is mediated by gap junctions and is therefore not blocked by the sodium-channel blocker, tetrodotoxin (TTX). Such activity may account for developmental events that are insensitive to TTX.

A third potential source of activity for the developing cortex is the subplate—a transient population of neurons in the mammalian cortex that serves as an intermediate target for thalamic afferents. Neurons in the subplate extend axons into the developing cortical plate and form synaptic contacts with newly differentiating cortical neurons (Shatz, 1996). Fourth, patterned activity to the brain can be driven by sensory (Katz and Shatz, 1996) or motor (Inglis et al., 2000) inputs. Many cellular mechanisms also drive spontaneous activity in the spinal cord (Milner and Landmesser, 1999).

In some cases, rather than decreasing dendritic growth, blocking synaptic activity paradoxically increases dendritic growth. For example, blocking glutamate receptors or L-type calcium channels increases dendritic growth of pyramidal and nonpyramidal neurons in cultured slices of ferret visual cortex (McAllister, 2000). Blocking NMDA receptors in neonatal ferrets in vivo also increases dendritic branching and spine formation in neurons in the lateral geniculate nucleus (Rocha and Sur, 1995). There are evidences that AMPA receptor activation can also limit dendritic growth (Feldmeyer et al., 1999).

In addition to a potent role for excitatory inputs, inhibitory inputs are also important in regulating dendritic growth (Sanes et al., 1992). Thus, the effects of neuronal activity on dendritic form may be site- and context-dependent (McAllister, 2000).

These paradoxical effects of decreased activity in enhancing dendritc growth are poorly understood (McAllister, 2000). Perhaps the simplest explanation for the widely differing effects of activity in these studies is that the cellular and molecular mechanisms of dendritic growth may differ between animal species and between brain regions (McAllister, 2000).

Many evidences suggest that defects in dendrite development and/or maintenance could contribute to neurological and neurodevelopmental disorders such as schizophrenia, Down's syndrome, fragile X syndrome, Angelman's syndrome, Rett's syndrome and autism (Miller and Kaplan, 2003; Bagni and Greenough, 2005; Pardo and Eberhart, 2007; Ramocki and Zoghbi, 2008; Walsh et al., 2008; Bourgeron, 2009).

Long-term confocal microscopy imaging in adult rodents indicates that a large fraction of dendritic spines as well as complete dendritic arbors are stable for extended time periods of several months, and possibly years (Lin and Koleske, ; Holtmaat et al., 2005; Majewska et al., 2006). Moreover, individual synapses may last for the majority of an organism's lifetime, possibly decades in the case of humans. Synapse and dendritic spine loss and dendritic atrophy are observed in the aging human brain (Lin and Koleske). Reductions in synapse number and dendritic arbor size are also associated with psychiatric illnesses, such as schizophrenia and major depressive disorder (MDD), as well as neurodegenerative diseases, such as Alzheimer's disease (AD) (Flood, 1991; Anderton et al., 1998; Law et al., 2004; Stockmeier et al., 2004; Broadbelt and Jones, 2008). These reductions in synaptic connectivity are believed to be a major contributor to the altered mood and impaired perception and cognition that characterize these conditions.

SYNAPSES

Synapses are specialized cell junctions through which neurons can communicate to each other (Purves, 2008).

There are two types of synapse: electrical and chemical. The first one is relatively rare and characterized by gap junction that let flowing of ionic current and secondary messengers across the two neurons. This structure allows a bidirectional transmission and a fast communication without the delay common in chemical synapses (Purves, 2008).

Chemical synapses are the most diffused type in the central nervous system. They are composed, in order, by a pre-synaptic compartment, a synaptic cleft and a postsynaptic compartment; moreover recent studies includes astrocytes in the synapse (Tamminga et al.) (Fig.b).

In this type of synapse, the communication is mediated by chemical agents, neurotransmitters, stored in vesicles of almost 40 nm diameter localized at the axon terminal of the presynaptic cell (Sheng and Hoogenraad, 2007). When an action potential in the presynaptic cell reaches an axon

terminal, it induces a localized rise in the level of Ca^{2+} in the cytosol. This, in turn, causes some of the vesicles to fuse with the plasma membrane, releasing their contents into the synaptic cleft, the narrow space between the cells (about 20-25 nm) (Lodish, 2000).

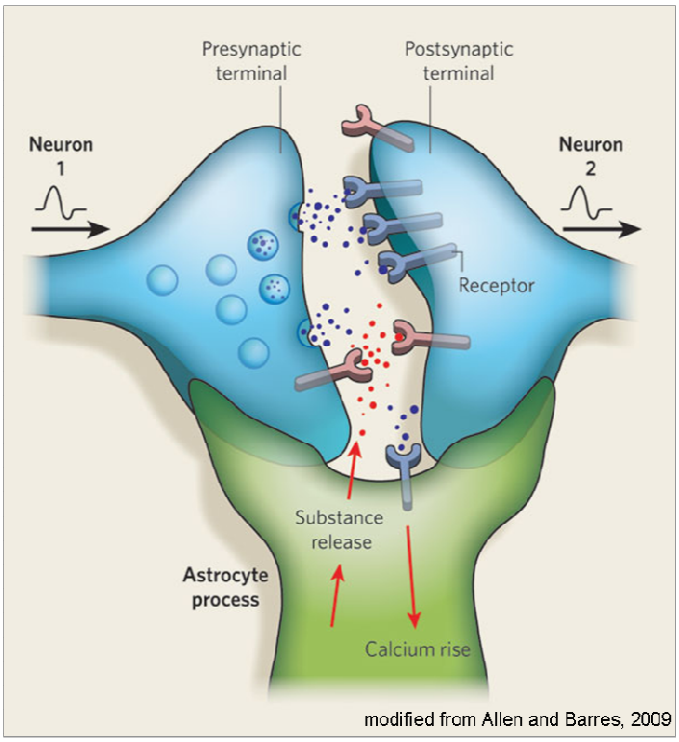


Fig. b. A tripartite synapse: When neurotransmitters are released from the presynaptic terminal of a neuron, astrocytic receptors are thought to be activated, leading to a rise in calcium ions in the astrocyte and the release of various active substances, such as ATP, which act back on neurons to either inhibit or enhance neuronal activity. Astrocytes also release proteins, which control synapse formation, regulate presynaptic function and modulate the response of the postsynaptic neuron to neurotransmitters (Allen and Barres, 2009).

The neurotransmitter (mainly glutamate for excitatory and GABA for inhibitory synapses in CNS) will reach and bind to the receptors on the post-synaptic membrane causing their opening and the incoming of ions (sodium and calcium for excitatory synapses or chlorum for inhibitory one) in the intracellular compartment. So, the effect on the post synaptic neuron (depolarization or iperpolarization) can determine the difference between excitatory and inhibitory synapse. Moreover excitatory synapses have a zone enriched of receptors to receive neurotransmitters, that is called post synaptic density (PSD) for its electron dense thickening of membrane, well visible at electron microscope, so defined asymmetric, instead inhibitory ones are defined symmetric for similar thickening of the pre e post-synaptic membrane (Sheng and Hoogenraad, 2007).

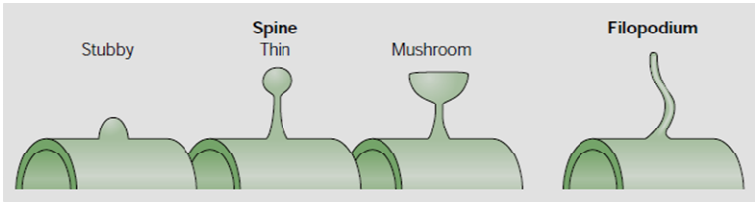
The presynaptic compartment is highly organized to allow the fusion of synaptic vesicles and the release of neurotransmitters in response to Ca^{2+} influx and presents a complex protein machinery to do so (Frank et al.). Synaptic vesicles consist of a lipid membrane with interspersed proteins that can enable Ca^{2+}-dependent responses for the release of neurotransmitters into the synaptic cleft. Among other things, synaptic vesicles must be trafficked to the presynapse, loaded with neurotransmitters, and docked at the site of release before fusion with the cell membrane. After the fusion with the cell membrane, synaptic vesicles must then be endocytosed to allow for the refilling with neurotransmitters and so another cycle of neurotransmitter delivery via synaptic vesicles can begin (Sudhof, 2004). Some of the proteins involved in this synaptic vesicles cycle and dynamics are Glutamate decarboxylase-65 (GAD65), vesicular glutamate transporter (VGLUT), vesicular gamma-Aminobutyric acid (GABA) transporter (VGAT), Synapsin (Syn), Synaptotagmin (SYT), Synaptophysin (Syp) and Cask. For example, GAD65 is involved in the (local) synaptic synthesis of GABA, whereas VGLUT and VGAT are involved in the loading of synaptic vesicles. Instead, Syns are involved in the anchoring of synaptic vesicles to the presynaptic scaffold, as well as the Ca^{2+}-dependent release of synaptic vesicles from the scaffold, Syp appears to be relevant for the endocytosis of synaptic vesicles, and adaptor proteins like Cask are indirectly involved in the anchoring synaptic vesicles at the presynaptic release site (Kwon and Chapman, ; Sudhof and Rizo, ; Kanaani et al., 2002; Tabuchi et al., 2002).

DENDRITIC SPINES AND PSD

Dendritic spines are small (typically 0.5–2 μm in length) actin-rich protrusions that house the essential excitatory postsynaptic components, including the post-synaptic density (PSD), actin cytoskeleton, and a variety of “supporting” organelles like mitochondria. Spines occur at a density of 1–10 spines per μm of dendrite length on principal neurons, and they receive most of the excitatory synapses in the mature mammalian brain (Sala and Segal).

Typical spines have a bulbous head (receiving a single synapse) connected to the parent dendrite through a thin spine neck.

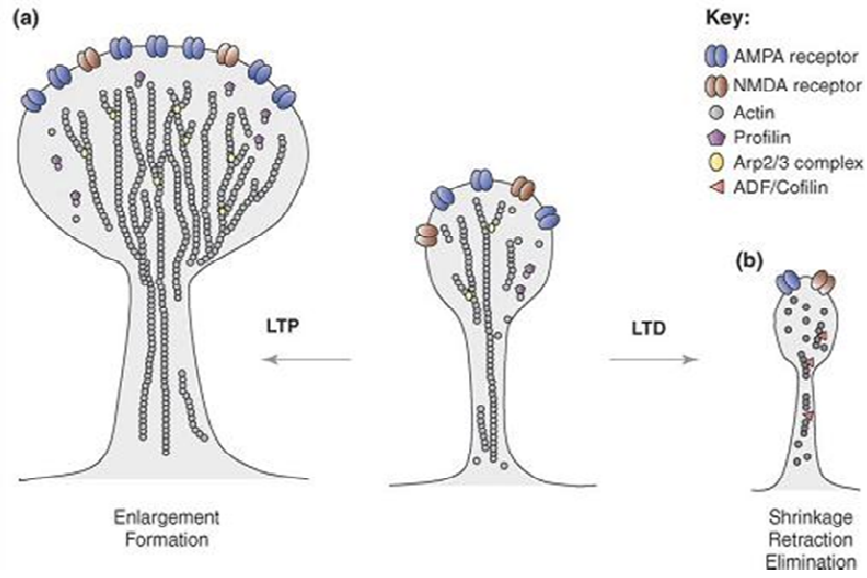
Dendritic spines are highly heterogeneous structures that show dynamic motility, especially during development. Their number, size, and shape undergo plastic changes correlated with long-term modifications of synaptic strength like certain form of synaptic plasticity (LTP and LTD) and interneuronal connectivity (Fig. d). Spine shape has been categorized as “mushroom,” “thin,” or “stubby,” (Sheng and Hoogenraad, 2007) but EM studies show a continuum between these categories (Fig. c).



modified from Yuste and Bonhoeffer, 2004

Fig. c. A schematic representation of filopodium and different types of dendritic spines (Yuste and Bonhoeffer, 2004).

Synapse formation and stabilization needs filopodia, structures present only in developing dendrites (or in adults ones, in pathological condition), distinguishable only on their morphological features. Filopodia is an exploratory shape for spines that can be determinant to the final form of spine. The aim of this structure is to explore and establish first contact with axons (Li and Sheng, 2003) (Yuste and Bonhoeffer, 2004). There are evidences that different spine shapes and sizes reflect different developmental stages and/or altered strength of synapses. Imaging experiments indicate that the volume of spine heads can increase with stimuli that strengthen synapses and can decrease with stimuli that weaken synapses. Spines with large heads are generally stable, express large numbers of AMPARs, and contribute to strong synaptic connections. By contrast, spines with small heads are more motile, less stable, and contribute to weak synaptic connections.



modified from Tada and Sheng, 2006

Fig. d. Changes in actin polymerization and spine morphology with LTP and LTD: (a) LTP (Long Term Potentiation) is associated with a shift of actin equilibrium toward F-actin (linear chains of monomeric G-actin) in spines, enlargement

of the spine head, and recruitment of more AMPA receptors to the postsynaptic membrane. (b) By contrast, LTD (Long Term Depression) stimulation shifts the equilibrium toward actin depolymerization, resulting in shrinkage or loss of spines (Tada and Sheng, 2006).

For instance, in studies on mouse brain, it is evident that a large fraction of mushroom spines are persistent, with lifetimes up to many months (Sheng and Hoogenraad, 2007). The size, shape, motility, and stability of dendritic spines depend largely on actin, the primary cytoskeleton within spines. A complex network of regulatory proteins, including the Rho family GTPases, controls actin arrangement and spine morphogenesis.

Since dendritic spines are thought to represent a morphological correlate of neuronal plasticity, altered spine morphologies may underlie or contribute to cognitive deficits seen in intellectual disability (von Bohlen Und Halbach).

The Post-Synaptic Density (PSD) is a specialized region at the excitatory post-synaptic compartment that is enriched in receptors, scaffold proteins and secondary messengers. PSD is important for signal transduction and synaptic plasticity (Sheng and Kim, 2002) (Fig. e).

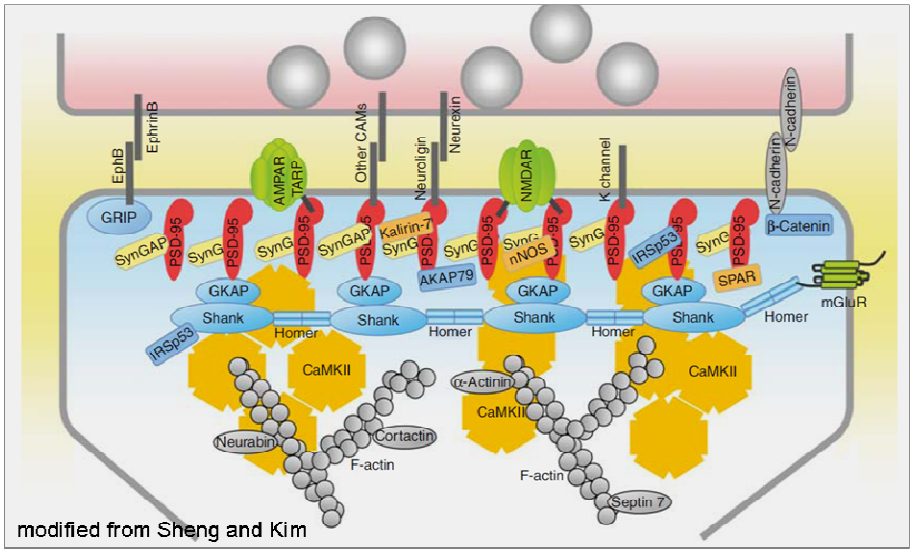


Fig. e. Molecular organization of the PSD of excitatory synapses: schematic diagram of the major proteins of the PSD, with protein interactions indicated by direct contacts or overlaps between the proteins (Sheng and Kim).

The postsynaptic compartment is highly organized to enable a response to neurotransmitter binding which includes (but is not limited to) a neurotransmitter-dependent influx of ions and a neurotransmitter-dependent induction of signaling cascades. For example, the postsynapse harbors

neurotransmitter receptors like the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), an ionotropic GluRs (Dingledine et al., 1999) (Sarto et al., 2002) and metabotropic neurotransmitter receptors like mGluRs which diversify the response of the postsynapse to neurotransmitters.

Additionally, the postsynapse exhibits an extensive scaffold which links neurotransmitters receptors with the cytoscelleton and signalling machinery; some prominent members of this scaffold include Postsynaptic Density protein 95 (PSD-95), Shank and Homer1.

Scaffold proteins are involved in the assembly of synaptic complex by linking different players by their protein-protein interaction domain. One of most studied family of scaffold protein in PSD is the MAGUK family (Membrane-associated Guanylate Kinase), that includes PSD-95 (o SAP90), SAP102, SAP 97, Chapsyn 110 (o PSD93) and p55 (Sala et al., 2001). The most studied MAGUK is PSD-95 because of its role in organization of signaling complexes at PSD. PSD-95 has the typical structure of MAGUK, including the three PDZ domains, an SH3 domain and a Guanylate Kinase dead domain (GK domain). PSD-95 can be a central organizer for PSD: it's involved in the recruitement of diverse protein to sites of synaptic adhesion, in promotion of trans-synaptic signaling and in coupling neuronal activity to changes in synaptic adhesion (Kreutz and Sala).

Another well-know family of scaffold protein massive present in PSD is ProSAP/Shank family. It consists by three members: Shank1, Shank2 and Shank3, all enriched in PSD and localized at the interface between membrane receptors and cytoskeletal elements (Kreutz and Sala). Shank proteins can interact directly or via adaptor proteins with glutamatergic receptors.

GKAP/SAPAP is a family of adaptor proteins that can bind Shank PDZ via their C-terminal domain and MAGUKs such as PSD-95 via their N-terminal domain. Shank1 and 3 can recruit Homer1b via their proline-rich domain; Homer1b, in turn, clusters with mGlu1 and 5 via its C-terminal and further interacts with IP3R (inositol triphosphate receptor) of the spine apparatus (Sala et al., 2001).

In the postsynaptic compartment there are also cell adhesion molecules like neuroligin-1 (NLGN1), which are linked to presynaptically located cell adhesion molecules like neurexins and, finally, the postsynaptic compartment also exhibits many signalling proteins, among them the most important are the Ca^{2+}/Calmodulin-dependent kinase II (CaMKII) and neuronal nitric oxide synthases (McAllister, 2002; Sfakianos et al., 2007; Satoh et al., 2008; Zheng et al., 2008).

DENDRITIC AND SYNAPSE ARCHITECTURE AND NEUROLOGICAL DISORDERS

Thanks to last years studies, it is clear that alterations in neuronal structure, like alterations in number or length of dendritic branches, or in number and shape of dendritic spines, are associated with intellectual disability (ID) or autism.

Given the functional relationship of PSD proteins to synapse development, structure, and function, it is not surprising that mutations in many PSD proteins are associated with human neurologic and psychiatric disease (Bayes et al.). Autism spectrum disorders have been linked to mutations in genes encoding Shank1, Shank2 and Shank3, PSD-93, DLGAP2/SAPAP2, and SynGAP1, as well as synaptic adhesion molecules neuroligin 3, neuroligin 4, and neurexin 1 (Berkel et al., ; Pinto et al., ; Durand et al., 2007; Sudhof, 2008). Mutations in SAPAP3 are associated with obsessive compulsive disorder (Welch et al., 2007; Zuchner et al., 2009).

Consistent with the necessity of appropriate dendritic architecture for higher order brain functions, including learning and memory, deficiencies in the architecture of dendrites have been observed in a variety of neurodevelopmental, neurodegenerative and neuropsychiatric disorders (Kulkarni and Firestein). For example, decreased dendritic branching in CA1 and CA4 hippocampal neurons occurs in patients with autism and neurons from patients with Rett syndrome. Similarly, altered dendritic arborization and decreased expression of glutamate receptors have been observed in CA3 hippocampal neurons of patients with schizophrenia (Kolomeets et al., 2007). Specific dendritic alterations are seen in cortical neurons in those with Rett syndrome. Decreased dendrite number have also been observed in patients with Alzheimer's Disease (Couch et al.), and Down Syndrome (Dierssen et al., 2003). In addition to the neurodevelopmental and neurodegenerative disorders, other neurological disorders may be associated with alterations in the dendritic arbor that compromise higher order functions, including cognition. For example, recurrent seizures during development in mice lead to a suppression of the growth of dendrites in the CA1 region (Casanova et al.) and may thus contribute to the cognitive deficits observed in childhood epilepsy. Thus, an abnormality in the dendritic arbor is a common theme seen in disorders of the central nervous system and these aberrations may be directly linked to deficiencies in higher order brain functions (Arikkath).

INTELLECTUAL DISABILITY

Intellectual disability (ID) affects about 2% of population of industrialized country. ID is defined by 3 main criteria: intelligent quotient lower than 70 (the mean in the population is 100), deficit in adaptive behavior, including autism spectrum disorders (ASD) in several patients and onset before 18 years old (Verpelli et al.).

ID can be caused by non-genetic factors, like infecions, prematurely birth, perinatal anoxia, etc., that represents almost 50% of cases, meanwhile the other cases are attributable to genetic factors.

Genetic causes are many and different; they can be grouped in three big panel: one includes genes involved in metabolic pathway common between different organs or cell type, the second comprises

defect in nervous system development with alteration in neuron interaction and the last includes all genes that causes only ID, named non-syndromic or non-specific ID. Moreover genes for ID can be associated to chromosome X or autosomic chromosome (Verpelli et al.).

More than 50% of the ID-related proteins that are not transcription or chromatin-remodeling factors, are clearly present in the pre- or post-synaptic compartments, implicated in synaptic functions by regulating synapse formation, actin cytoskeleton rearrangement (i.e. OPHN1, PAK3, and ARHGEF6), or synaptic plasticity. The synapse-related proteins associated with ID can be separated into two groups, one that localizes fully at synapses and whose deletions and mutations directly impede with synaptic formation, and a second cluster that indirectly controls neuronal development and synapse formation by regulating synthesis and degradation of major synaptic proteins or the synaptic actin cytoskeleton assembly or disassembly. Another recurrent feature is the high expression of altered gene in hippocampus, a brain region strongly involved in learning and memory (Verpelli et al.).

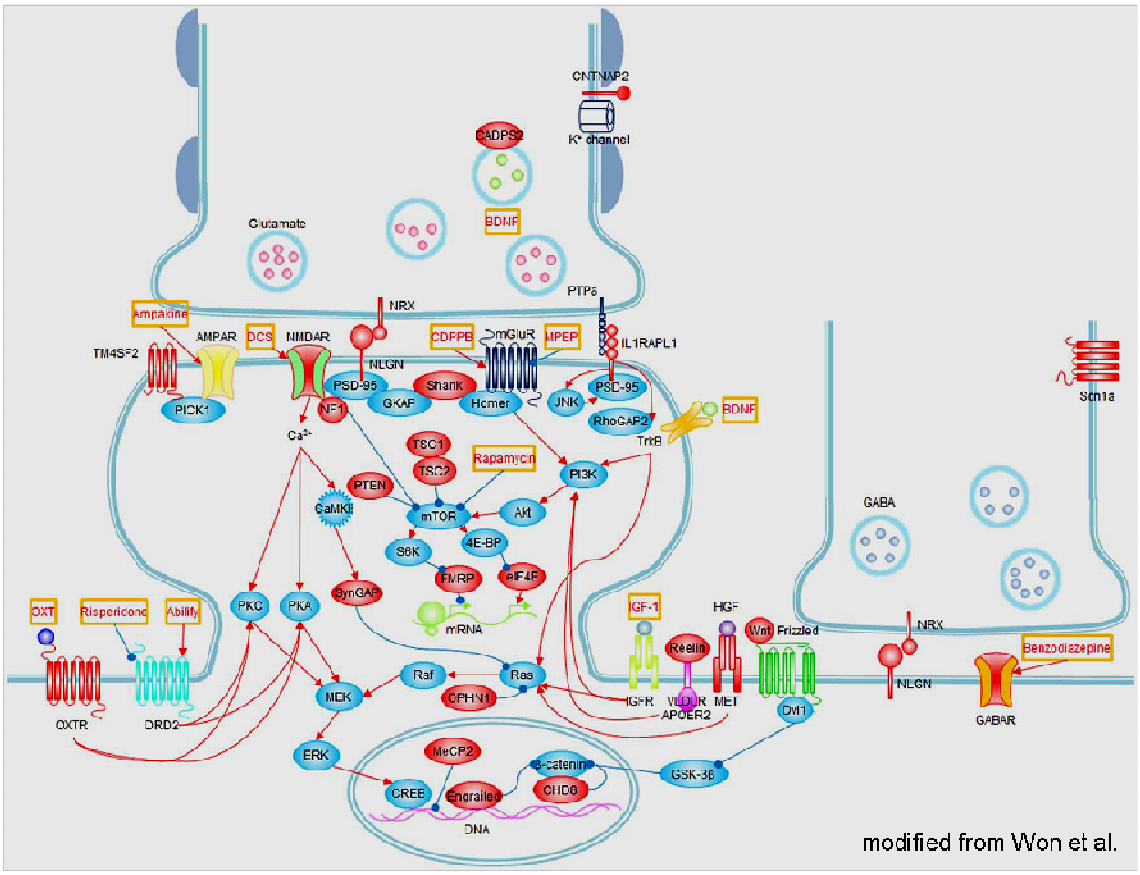


Fig. f. Signaling pathways and possible treatments associated with ASD. Molecules whose mutations or polymorphisms are associated with ASD are indicated in red. Stimulations and inhibitions are indicated by red and blue arrows, respectively. Possible treatments and their target molecules are indicated by red texts in orange boxes. SynGAP1,which directly interacts with PSD-95, could not be placed next to PSD-95 for simplicity (Won et al.).

Unfortunately, only two medicines are currently approved for ASD by US FDA; risperidone (Risperdal®) and aripiprazole (Abilify®), which act as dopamine/5-HT receptor antagonists (McPheeters et al.). These drugs are useful for correcting irritability and stereotypy, but not sociability defects. Recently, a number of candidate ASD medications for treating social abnormalities have been suggested (Won et al.) (Fig. f).

Positive allosteric modulators of mGluR5 receptors were first developed to alleviate symptoms of schizophrenia (Gregory et al.) augmenting NMDAR signaling via mGluR potentiation (Stefani and Moghaddam, ; Uslaner et al., 2009). A large number of mGluR5 allosteric modulators have been developed of these, CDPPB, in particular, has been examined in various behavioral assays and model animals (Stefani and Moghaddam, ; Won et al., ; Kinney et al., 2005; Uslaner et al., 2009). These published results suggest that mGluR5 positive allosteric modulators have the potential to improve cognitive impairments associated with brain disorders including schizophrenia and autism.

D-cycloserine is a high-affinity partial agonist of NMDA-coupled, strychnine-insensitive glycine receptors. Putative effects of D-cycloserine on ASD have been suggested by previous studies (Blundell et al., ; Posey et al., 2004). Moreover, D-cycloserine partially rescues social deficits of Shank2−/− mice, supporting the role of NMDAR functionality in autism (Won et al.).

Clonazepam, a type of benzodiazepine, is a positive allosteric modulator of GABAA receptors that exerts sedative, hypnotic, anxiolytic, anticonvulsant, and muscle relaxing effects (Rudolph and Knoflach). Similar to the action of mGluR positive allosteric modulators, clonazepam potentiates GABA signaling only when GABAA receptors are bound by their endogenous ligand, GABA. Published results indicate that Clonazepam is able to normalize disrupted E-I balance in ASD mouse models and that is a promising strategy for treating symptoms of ASD (Won et al.).

Synaptic protein synthesis is stimulated by local mRNA translation, a process that depends on group I mGluR activation. FMRP, encoded by the Fmr1 gene, is a repressor of mRNA translation; thus, mGluR-mediated protein synthesis could be enhanced in the absence of FMRP. Therefore, attempts have been made to correct fragile X syndrome by suppressing abnormally enhanced mGluR5-dependent synaptic plasticity and protein synthesis (Yan et al., 2005). In line with this, mGluR negative allosteric modulators are now in clinical trials for fragile X syndrome patients (Krueger and Bear).

Rett syndrome is an X-linked neurological disorder caused by mutations in the MeCP2 gene. MeCP2 is a transcriptional repressor and activator, which binds widely across the genome and influence a large number of genes (Chahrour et al., 2008). One of the best characterized targets of

MeCP2 is BDNF, a neurotrophic factor that regulates neuronal development and synaptic plasticity (Greenberg et al., 2009). Moreover, restoring Bdnf expression through ampakine administration alleviates respiratory problems of MeCP2 mutant mice (Ogier et al., 2007). Another growth factor associated with Rett syndrome is insulin-like growth factor 1 (IGF-1). IGF-1 is a polypeptide hormone with structural similarity to insulin. While it has a profound effect on overall cell growth, it also plays an important role in regulating neuronal functions by promoting axonal outgrowth (Ozdinler and Macklis, 2006), neuro- and synaptogenesis (O'Kusky et al., 2000), and activity-dependent cortical plasticity (Tropea et al., 2006). IGF-1 activates Ras-MAPK and PI3K-Akt pathways (Fernandez et al.), signaling cascades that are also activated by BDNF. The therapeutic utility of IGF-1 in Rett syndrome was originally suggested by Mriganka Sur and coworkers, who reported that lethality, hypoactivity, and respiratory problems of MeCP2-null mice are partially rescued by IGF-1 treatment in association with normalization of impaired spine density, synaptic transmission, and cortical plasticity (Tropea et al., 2006). IGF-1 also reverses the reduction in excitatory synapse number and density of neurons derived from Rett patients (Marchetto et al.).

Rapamycin is an immunosuppressant that strongly binds to FK506-binding protein (FKBP); this complex then binds and inhibits mTOR, a serine/threonine kinase implicated in transcription, cytoskeleton dynamics, ubiquitin-dependent protein degradation, autophagy, and membrane trafficking (Dennis et al., 1999). Perturbations in mTOR signaling have significant impacts on normal brain functions. The therapeutic utility of rapamycin in ASD was suggested in 2008 based on studies in Tsc2+/− mice (Ehninger et al., 2008). The mTOR pathway is associated with TSC because TSC1 and TSC2 are upstream inhibitory regulators of mTOR activity (Han and Sahin). In this study, the learning and memory deficits, lethality, aberrant brain overgrowth, and altered synaptic plasticity of Tsc2+/− mice were ameliorated by acute treatment with rapamycin. The social dysfunction and behavioral inflexibility of Purkinje cell-specific Tsc1 mutant mice were also improved by rapamycin (Tsai et al.), further suggesting that rapamycin may be useful in reversing core symptoms of autism.

Oxytocin is associated with various social behaviors including affiliation, maternity, aggression, and pair bonding (Caldwell, ; Feldman). Given the prominence of oxytocin in the regulation of social behavior, the association of oxytocin with autism pathogenesis has been extensively examined. Several SNPs of OXTRs are associated with ASD (Yrigollen et al., 2008) (Liu et al.). Oxtr knockout mice display autistic-like behaviors; they emit fewer USVs upon social isolation, show defects in social recognition and discrimination, and are less aggressive (Crawley et al., 2007). Supporting the pharmacotherapeutic potential of oxytocin, nasal administration of oxytocin improves social interactions and communications (Andari et al., ; Kosaka et al.), reduces repetitive

behaviors (Hollander, ; Hollander et al., 2003), and enhances social cognition (Hollander et al., 2007) in autism-affected individuals.

Moreover, NMDAR antagonists including amantadine and its close analogue memantine are now in clinical trials for autistic patients (Spooren et al.).

Severe forms of ID are often attributable to mutations of a single gene on the X chromosome (Verpelli et al., ; Chechlacz and Gleeson, 2003). X-linked ID was first discovered in 1938 by Penrose: analyzing 1280 patients institutionalized, he saw a higher percentage of male with ID (20-25%) than female. The association with chromosome X was definitively confirmed in 1970 by Lehrke using genetic mapping (Lehrke, 1972; Carter, 2002).

The majority of the XLID are attributable to the Fragile X and Rett syndromes, however deletions and mutations of several other genes on chromosome X have been found strongly associated with ID (Verpelli et al.). Interleukin-1 receptor accessory protein like 1 (IL1RAPL1) gene is localized on X chromosome and a number of mutations in this gene (IL1RAPL1; OMIM 300206) have been found in patients with cognitive impairments ranging from non syndromic ID to ASD (Barone et al., ; Behnecke et al., ; Franek et al., ; Koh et al., ; Mignon-Ravix et al., ; Youngs et al., ; Carrie et al., 1999; Bahi et al., 2003; Tabolacci et al., 2006; Piton et al., 2008). There is also a case of startle epilepsy associated with *IL1RAPL1* gene deletion (Dinopoulos et al.).

IL1RAPL1 AND ITS INTERACTORS

The IL1RAPL1 protein belongs to a new Toll/IL-1 receptor family and shares 52% homology with the IL-1 receptor accessory protein (IL-1RacP) and it is structurally formed by three extracellular Ig-like domains, a transmembrane domain, and an intracellular Toll/IL-1R homology domain (TIR domain). *Il1rapl1* gene extents for 1.3 Mbases on chromosome X in Xp22.1–21.3 region (Carrie et al., 1999). cDNA is composed by 11 exons for 3,6 Kbasi total (NM_014271).

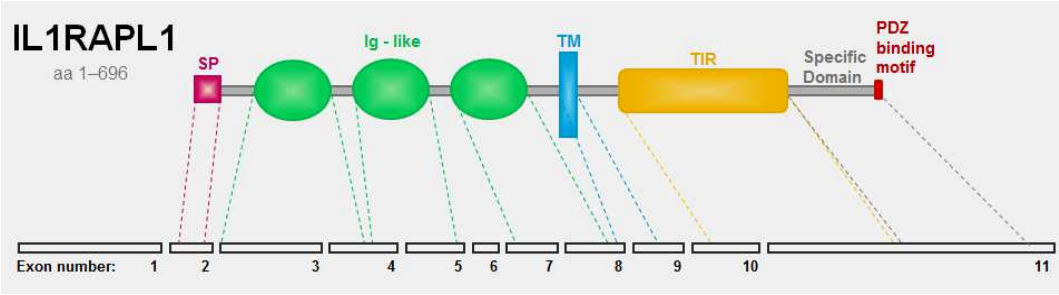


Fig. g. Schematic representation of IL1RAPL1 domains: the signal peptide (SP), the three IG-like domains, the transmembrane segment (TM), the TIR domain, the specific domain of IL1RAPL1 and the putative PDZ binding motif.

Il1rapl1 gene encodes for a transmembrane protein of 696 aminoacids with a molecular weight of approximately 80 KDa. IL1RAPL1 has a signal peptide of 18 aa with a cleavage site at TQS/LK predicted site and a 17 amino acid transmembrane segment that divides the protein into two domains: a 359 amino acid extracellular and a 319 amino acid cytoplasmic domain. The extracellular domain is rich in cysteine residues that are responsible for formation of three Ig-like domains (Fig. g).

Another member of this class is IL1RAPL1 homologue, IL1RAPL2 (or TIGIRR-1, three immunoglobulin domain-containing IL-1 receptor-related) that shares 63% amino acid identity with IL1RAPL1 (Born et al., 2000).

The TIR domain, typical of human superfamily of IL-1/Toll receptor, has 150-200 conserved aminoacid residues and it is important to mediate protein-protein homophilic interaction in innate immunitary response pathway (Radons et al., 2003). Although the homology, IL1RAPL1 and IL1RAPL2 seem not to be involved in inflammatory response (NF-kB-dependent signaling pathway) and are unable to bind any isoform of IL-1 (Born et al., 2000).

IL1RAPL1 transcripts are expressed at a low level in fetal and adult brain: the first detectable transcript in mouse brain is at E10.5 with an increased level at E12.5 that remains the same in adult life. The high level of expression of *IL1RAPL1* in brain areas that are involved in memory development, such as hippocampus, dentate gyrus and entorhinal cortex, suggests that this gene may have a specialized role in physiological processes underlying memory and learning abilities (Carrie et al., 1999; Gao et al., 2008). It is also highly expressed in mouse olfactory bulb and tubercule, which may be correlated with the evolutionary trend implicating the predominant role of olfactory perception in behavioural development in lower mammals (Carrie et al., 1999). Analysis of the other tissues reveal a transcription of *IL1RAPL1* in heart, brain, ovary, skin, and a lesser level of expression in tonsil, fetal liver, prostate, testis, small intestine, placenta, and colon. Expression was not detected in spleen, lymphnode, thymus, bone marrow, leukocytes, lung, liver, skeletal muscle, kidney, or pancreas (Born et al., 2000). *IL1RAPL1* expression is very low compared with that of *VGLUT1* or *GAD67* (Houbaert et al.). Higher expression levels of *IL1RAPL1* mRNA were recurrently seen in olfactory bulbs and in dentate gyrus of the hippocampus. In the amygdaloid complex, expression spans all excitatory (basolateral amygdala) and inhibitory (intercalated cells and central amygdala) regions homogeneously (Houbaert et al.).

The mouse homologue of human *IL1RAPL2*, is mainly expressed in skin and liver (Born et al., 2000). Although the low level expression of *IL1RAPL2* in brain, it has a complementary pattern of expression and is mainly expressed in the cingulum, colliculus and lateral substantia nigra. These

results suggest that both genes may have similar functions, but in different brain structures (Bahi et al., 2003).

The level of expression of *IL1RAPL*, was significantly up-regulated following kainite treatment and LTP induction, suggesting a possible role for this gene in activity-dependent brain plasticity (Boda et al., 2002).

About IL1RAPL1 protein localization in synapse, immunocytochemical staining with anti-IL1RAPL1 antibody shows that endogenous protein clusters are distributed in the dendrites of cultured cortical neurons and partially overlapped with postsynaptic Shank1 clusters (Pavlowsky et al.); meanwhile the overexpression of IL1RAPL1 increases the staining signals of excitatory postsynaptic marker Shank2 (Yoshida et al.). A comparison between Shank proteins clusters signal and synaptophysin (a marker of presynaptic compartment) clusters signal underlines a predominant postsynaptic localization of IL1RAPL1 (Pavlowsky et al.). Moreover there is a high level of colocalization between IL1RAPL1 and PSD-95, instead it is only partial with VGAT (a marker of inhibitory synapse), so IL1RAPL1 is mostly localized in excitatory synapses (Pavlowsky et al.).

In vitro, IL1RAPL1 plays a role in presynaptic differentiation and dendritic spine formation and stabilization in cortical and hippocampal neurons. siRNA against IL1RAPL1 can reduce number of dendritic protrusion in neuron culture (Yoshida et al.) and IL1RAPL1 overexpression in neurons increases VGLUT1 (an excitatory presynaptic marker) staining and dendritic spine number (but not the length or the width of the heads) (Pavlowsky et al., ; Valnegri et al.). Furthermore, the increase in synapse number is associated with an increase of miniature excitatory postsynaptic potential (mEPSC) frequency (Pavlowsky et al.).

In vivo, the role of *IL1RAPL1b*, an orthologous of *IL1RAPL1*, was analyzed in olfactory sensory neurons of zebrafish. Antisense morpholino oligonucleotide against *IL1RAPL1b* suppressed both synaptic vesicle accumulation and axon terminal remodeling. Consistently, the overexpression of *IL1RAPL1b* stimulated synaptic vesicle accumulation. Swapping the carboxyl-terminal domain of *IL1RAPL1b* with that of mouse IL-1 receptor accessory protein abolished the stimulatory effect. On the other hand, a substitution mutation in the TIR domain suppressed the morphological remodeling of axon terminals. Thus, the regulation of synaptic vesicle accumulation and subsequent morphological remodeling by IL1RAPL1b appeared to be mediated by distinct domains (Yoshida and Mishina, 2008).

In mouse model, the loss of IL1RAPL1 in KO mice lead to a slight but significant reduction of spine density, especially in the cortex (Yasumura et al.) and in the CA1 region of hippocampus (Pavlowsky et al.), meanwhile the architecture of this contacts is unchanged (spine shape,

presynaptic structure, …) and there isn't any difference in number or organization of inhibitory synapse (Pavlowsky et al.). Functionally, the reduction of excitatory synapse number results in a little, not significant reduction of mEPSC and LTP impairment only with a paradigm of theta burst stimulation (LTP for high frequency stimulation is comparable to WT mice). Data of behavioral studies shows that IL1RAPL1 KO male mice show deficits in spatial memory (Pavlowsky et al.) and altered cued fear memory formation (Houbaert et al.). In addition, the acquisition and retention of spatial reference memory, the spatial working memory and long-term fear memories are impaired in IL1RAPL1 KO mice (Yasumura et al.). Social interaction is increased in IL1RAPL1 KO mice and their motor coordination is improved, but the motor learning ability is comparable with WT mice (Yasumura et al.). Finally, IL1RAPL1 KO mice show enhanced locomotor activity and reduced anxiety-like behaviours (Yasumura et al.).

Abnormalities in the formation and function of cerebellar circuitry potentially contribute to cognitive deficits in humans. In *IL1RAPL1*-deficient mice the absence of IL1RAPL1 causes a transient disinhibition of deep cerebellar nuclei neurons and upstream, in the cerebellar cortex, developmental perturbations have been found in the activity level of molecular layer interneurons. Thus, IL1RAPL1 exerts a key function during cerebellar development in establishing local excitation/inhibition balance (Gambino et al., 2009). Interestingly, it was seen that more than 90% of autistic patients exhibit cerebellar abnormalities (Piton et al., 2008) thus this results provide an additional insight into physiological defects in patients.

In contrast to the other members of the Toll/IL-1 receptor family, IL1RAPL1 has 150 additional amino acids at the C-terminus, that interact with the neuronal calcium sensor-1 (Bahi et al., 2003), thus regulating type voltage-gated calcium channel activity in PC12 cells and in neurons (Gambino et al., 2007).

The stable expression of IL1RAPL1 in PC12 cells induces a specific silencing of N-type voltage-gated calcium channels (NVGCC) activity that explains a secretion deficit observed in these cells. Importantly, this modulation of VGCC activity is mediated by NCS-1. Indeed, a specific loss-of-function of N-VGCC was observed in PC12 cells over-expressing NCS-1, and a total recovery of N-VGCC activity was obtained by a down-regulation of NCS-1 in IL1RAPL1 cells. Because both proteins are highly expressed in neurons, these results suggest that IL1RAPL1-related ID could result from a disruption of N-VGCC and/or NCS-1-dependent synaptic and neuronal activities (Gambino et al., 2007).

Our laboratory showed that the first and second PDZ domains of PSD-95 bind to the C-terminal tail of IL1RAPL1 that is characterized by a PDZ putative binding motif (Pavlowsky et al.). Using gain- and loss-of-function experiments in neurons, we demonstrated that IL1RAPL1 regulates the

synaptic localization of PSD-95 by controlling c-Jun terminal kinase (JNK) activity and PSD-95 phosphorylation (Pavlowsky et al.).

IL1RAPL1 over-expression in hippocampal primary cultured neurons increases the spines number without affecting morphology (Pavlowsky et al., ; Valnegri et al.). This effect requires both extracellular and intracellular domains of IL1RAPL1 and it is not dependent on PSD-95 interaction: in hippocampal primary cultured neurons over-expressing IL1RAPL1Δ8 (IL1RAPL1 deleted mutant lacking the PSD-95 binding domain (Pavlowsky et al.)) the effect on spines is still present and the over-expression of IL1RAPL1ΔC, lacking most of the C-terminal region (Carrie et al., 1999), or ΔN (Behnecke et al., ; Carrie et al., 1999) results in a spine number similar to the control, demonstrating the role of both N-term and C-term regions of IL1RAPL1 in spine formation (Pavlowsky et al., ; Valnegri et al.). Interestingly, the extracellular domain is sufficient to increase excitatory pre-synaptic compartment recruitment (intensity level of VGLUT1 staining) instead the inhibitory pre-synaptic excitatory staining (using VGAT as marker) is unchanged. Similar data was shown for IL1RAPL2 overexpression (Valnegri et al.).

These data suggest that multiple interactors are needed to induce spine formation. The C-terminal tail of IL1RAPL1, in particular TIR domain and the last 150 aa, can recruit RhoGAP2, meanwhile the extracellular domain can bind to receptor tyrosine phosphatase δ (PTPδ), a member of LAR-RPTP family, suggesting a role of IL1RAPL1 and IL1RAPL2 as synaptic cell adhesion proteins (Valnegri et al.).

RhoGAP2 interaction with IL1RAPL1 has been demonstrated in our laboratory through a yeast two hybrid assay on a human fetal brain cDNA library (Valnegri et al.).

RhoGAP proteins increase the intrinsic GTPase activity to inactivate the RhoGTPase switch and guanine-nucleotide dissociation inhibitors (GDIs) (Sala and Segal, ; Jaffe and Hall, 2005). Rho proteins have been implicated in different aspects of neuronal morphogenesis, including dendritic arbor development and spine morphogenesis: for example, Rac1 positively regulates and RhoA negatively regulates dendritic spines morphogenesis (Sala and Segal, ; Govek et al., 2004).

RhoGAP2 is expressed in the cortex, the cerebellum and the hippocampus of adult mice and in the cortex and hippocampus in mice during development. RhoGAP2 is also present in the synaptosomal fraction. Endogenous RhoGAP2 mostly co-localizes with PSD-95 and Shank1 proteins which are markers of excitatory post-synapses. The over-expression of RhoGAP2 in hippocampal neurons primary culture induces both excitatory synapse and dendritic spine formation. However, the over-expression of a RhoGAP2 mutant that does not bind to IL1RAPL1 causes a drastic change in spine shapes which are converted to filopodia like shape. Moreover, the endogenous RhoGAP2 staining is increased at synapses when IL1RAPL1 is over-expressed (Valnegri et al.).

The interaction of IL1RAPL1 with RhoGAP2 is required to induce dendritic spine formation. Interestingly, we found that blocking the IL1RAPL1/PTPδ interaction abolished RhoGAP2 recruitment at excitatory synapses, suggesting that IL1RAPL1 is involved in a novel *trans*-synaptic signaling pathway that regulates excitatory synapse and dendritic spine formation (Valnegri et al.) (Fig. h).

We also showed that the extracellular domain of IL1RAPL1 induces excitatory synapse formation by binding PTPδ, which is localized at the pre synaptic terminal (Valnegri et al.). Interestingly it was also demonstrated that the extracellular domain of IL1RAPL1 interacts only with particular splice variants of PTPδ (Yoshida et al.).

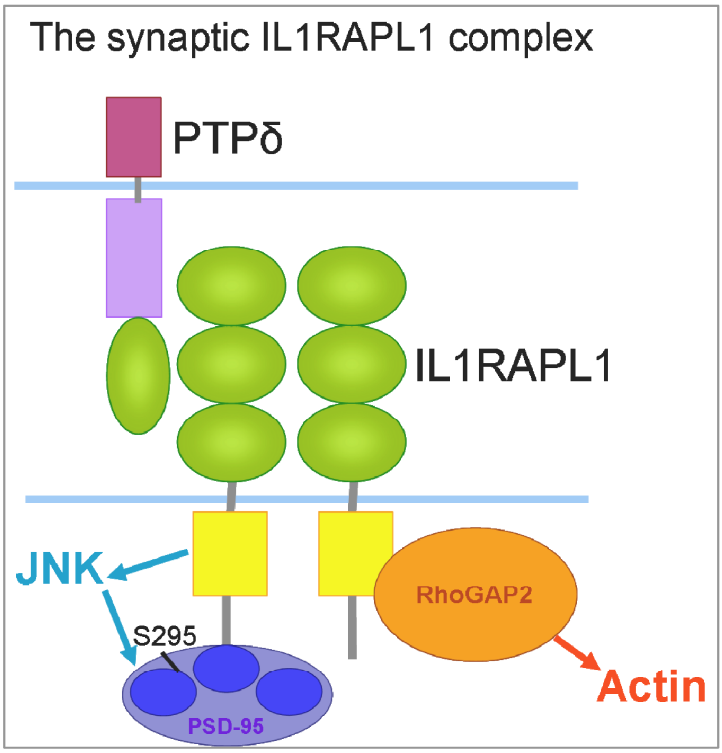


Fig. h. The synaptic IL1RAPL1 complex (Carlo Sala)

PTPδ is a member of LAR-RPTPs family, a subfamily of receptor protein tyrosine phosphatase (RPTP) type IIa, localized on synaptic membrane with a role in axon outgrowth and guidance (Dunah et al., 2005; Sajnani et al., 2005; Yang et al., 2005).

Mutations in chromosome locus of PTPδ (9p24.3-p23) have been associated with restless legs syndrome (Schormair et al., 2008), cancer (Hendriks and Pulido, ; Solomon et al., 2008), autism spectrum disorder (Pinto et al.), attention-deficit hyperactivity disorder (Elia et al.), bipolar disorder and schizophrenia (Malhotra et al.).

PTPσ and LAR are the other vertebrate members of LAR-RPTPs family. LAR-RPTPs are encoded by three independent genes and they share 66% of amino acid identity (Pulido et al., 1995).

LAR-RPTPs are composed by three cell adhesion immunoglobulin-like (Ig) and numerous fibronectin III (FNIII) domains (maximum eight), suggesting a role of LAR-RPTPs in cell-cell and cell-matrix interactions and therefore in synaptic adhesion and synapse organization. The intracellular region is composed by two protein tyrosine phosphatase (PTP) domains, one, the proximal to membrane, with a strong catalytic activity (D1), the other with a residual or no catalytic activity (D2) (Pulido et al., 1995; Wallace et al., 1998; Blanchetot et al., 2002; Gonzalez-Brito and Bixby, 2006). It was hypothesized that LAR-RPTPs exist as dimers when inactive and monomerize on ligand binding, resulting in activation of PTP (Um and Ko). LAR-RPTPs can interlink distinct tyrosine phosphorylation signaling pathways, mostly by dephosphorylating elements of these pathways. For example there is an interaction between PTPσ D1 domain and PTPδ D2 domain that has a negative regulatory function (Wallace et al., 1998).

LAR-RPTPs are expressed in different tissue; in brain, they are involved in trans-synaptic (both excitatory and inhibitory) complexes with three general functions in synaptic organization. One is to mediate cell–cell adhesion at synapses. For instance, the full-length extracellular domain (ECD) of PTPδ functions as a ligand to promote cell adhesion and neurite outgrowth (Wang and Bixby, 1999; Gonzalez-Brito and Bixby, 2006). The second one is to mediate presynaptic differentiation, local recruitment of synaptic vesicles and release and recycling machinery (a form of retrograde synaptogenic signaling triggered by binding of the postsynaptic partner to axonal RPTPs). The third is to trigger postsynaptic differentiation, local recruitment of neurotransmitter receptors, scaffolds, and signaling proteins (a form of anterograde synaptogenic signaling triggered by binding of the presynaptic RPTP to dendritic binding partners) (Takahashi and Craig, ; Um and Ko).

In situ hybridization of mice brain samples revealed that PTPδ mRNA is present in the hippocampus, thalamic reticular nucleus, and piriform cortex, where some Src family PTKs have been also demonstrated to exist (Mizuno et al., 1993). In adult mice brain, PTPσ and PTPδ show substantial levels of expression, whereas LAR staining is hardly detectable above background levels. A distinct higher expression of PTPδ is apparent in the hippocampal CA2, CA3 region and in dentate gyrus (Kwon et al., ; Schaapveld et al., 1998; Uetani et al., 2000). In the cerebellum, all three transcripts are present in the granular cell layer. In the olfactory bulb, low levels of LAR expression are observed in the internal granular layer and the mitral cell layer. In contrast, high levels of PTPσ transcripts are not only present in these layers but also in the glomerular cell layer. Strikingly, PTPδ is restricted to the mitral and glomerular cell layers only (Schaapveld et al., 1998). PTPδ is also expressed in B lymphocytes and thymic medulla (Uetani et al., 2000).

Structurally, LAR-RPTPs undergo constitutive proteolytic processing, that generate an extracellular subunit that remains non-covalently bound to the transmembrane region of the phosphatase domain subunit (Takahashi and Craig, ; Wang and Bixby, 1999).

This proteolytic cleavage takes place at a site in the ECD located near the membrane (Pulido et al., 1995) and the protein is expressed on the cell surface as a complex of two noncovalently associated subunits of,140 and 85 kDa (Wang and Bixby, 1999) .

Rabbit polyclonal antibody D5013 (Dunah et al., 2005) against the phosphatase domain D1 of human LAR-a domain that is highly conserved among LAR-RPTPs (all the three members of the LAR subfamily) was used on immunoblots of COS-7 cells transfected with LAR, PTPδ and PTPσ. D5013 antibody reacted with multiple species, including a top band of ~220–230 kDa that presumably corresponds to the full-length protein (LAR, 220 kDa; PTPδ, 220 kDa; and PTPσ 230 kDa) and multiple smaller bands that probably arise from proteolysis of LAR-RPTPs, as no signal was observed in untransfected COS-7 cells (Dunah et al., 2005). In rat cerebral cortex, the LAR antibody recognized a major band of Mr ~85 kDa, which is likely to represent the P-subunit containing the transmembrane and intracellular regions of LAR, PTPδ and/or PTPσ. The ~220 kDa LAR polypeptide shifted to a lower molecular weight upon treatment with glycosidase, supporting the idea that it represents the full-length protein. The ~85 kDa band was unaffected, consistent with it being the P-subunit of LAR-RPTP(Dunah et al., 2005).

Immunoblotting showed that LAR-RPTP proteins were widely expressed in different regions of the rat CNS, and at higher levels in postnatal day 7 (P7) brain than in adult (P45) brain, consistent with mRNA expression data. The ~85 kDa LAR-RPTP band showed the biochemical fractionation pattern expected for an integral membrane protein. LAR-RPTP was enriched in postsynaptic density (PSD) fractions, though to a lesser extent than PSD-95. At 7 d in vitro (DIV7), LAR-RPTP showed a fine punctate immunostaining in cell bodies and dendrites of cultured hippocampal neurons and only partial colocalization with PSD-95 or with bassoon, a protein expressed in the presynaptic active zone. With neuronal maturation, an increasing fraction of LAR puncta became synaptically localized, and an increasing percentage of synapses showed detectable LAR staining. The immunocytochemical data and biochemical findings are consistent with LAR-RPTP being enriched at excitatory synapses but more widely distributed in neurons than PSD-95 (Dunah et al., 2005).

Neurons expressing any single LAR-RPTP RNAi, or any combination of them, showed a lower density of spines and PSD-95 puncta than did vector-transfected cells. The LAR-RPTP RNAi–transfected neurons also showed a substantial decrease in the surface staining intensity of AMPA receptor subunit GluR2 (Dunah et al., 2005).

Moreover, it was found that PTPδ-deficient mice were semi-lethal due to insufficient food intake. They also exhibited learning impairment in the Morris water maze, reinforced T-maze and radial arm maze tasks. Interestingly, although the histology of the hippocampus appeared normal, the magnitudes of long-term potentiation (LTP) induced at hippocampal CA1 and CA3 synapses were significantly enhanced in PTPδ-deficient mice, with augmented paired-pulse facilitation in the CA1 region. Thus, it was shown that PTPδ plays important roles in regulating hippocampal LTP and learning processes, and that hippocampal LTP does not necessarily positively correlate with spatial learning ability (Uetani et al., 2000).

RPTPs modulate their effects on cells also interacting with proteoglycans. Heparan and chondroitin sulfate proteoglycans (HSPGs and CSPGs, respectively) regulate numerous cell surface signaling events, with typically opposite effects on cell function (Coles et al., ; Milev et al., 1994; Garwood et al., 2003).

Phosphacan is a CSPGs produced by glial cells in the central nervous system, and represents the extracellular domain of a receptor-type protein tyrosine phosphatase (RPTP zeta/beta). Binding to neural cell adhesion molecules, and possibly also by competing for ligands of the transmembrane phosphatase, phosphacan may play a major role in modulating neuronal and glial adhesion, neurite growth, and signal transduction during the development of the central nervous system (Milev et al., 1994). Moreover, CSPGs inhibit nerve regeneration through RPTPσ: RPTPσ acts bimodally in sensory neuron extension, mediating CSPG inhibition and HSPG growth promotion. In summary, proteoglycans can exert opposing effects on neuronal extension by competing to control the oligomerization of a common receptor (Coles et al.).

All the members of LAR-RPTPs family have a specific partner, different for each one, according to the possibility of selecting a molecular partner by insertion or exclusion of mini exons (me), short peptide of 4-16 aa. There are four mini exons, called meA, B, C and D (Yoshida et al., ; Pulido et al., 1995). meA and meB peptides are particular important by their localization into Ig domains: meA is predicted to affect the length of a loop region between the D and E β-strands of the second Ig-like domain, meanwhile the meB is predicted to affect the spacing between the second and third Ig-like domains. This feature is crucial to regulate the binding-affinity (Yoshida et al., ; Pulido et al., 1995).

The postsynaptic binding partners of LAR-RPTPs in trans-synaptic complexes have been identified as follows: netrin-G ligand-3 (NGL-3), neurotrophin receptor tropomyosin-related kinase C (TrkC), IL1RAPL1, interleukin-1 receptor accessory protein (IL1RAcP), and Slit and NTRK-like family (Slitrk 1–6). NGL-3 binds to LAR, PTPσ, and PTPδ through their first two FNIII domains (*in*

vitro). TrkC binds selectively to PTPσ, IL1RAPL1 selectively to PTPδ, IL1RAcP to LAR, PTPσ, and PTPδ, and Slitrks selectively to PTPδ (*in vitro*) and PTPσ, through the Ig domains of the RPTPs (Takahashi and Craig) (Fig. i).

Comparison between members of IL-1/Toll receptor family involved in synapse maturation (i.e. IL1RAPL1 and IL1RAcP) and binding RPTPs underlines their specific localization: IL1RAPL1 induces only excitatory presynaptic differentiation whereas IL1RAcP induces not only excitatory but also some inhibitory presynaptic differentiation. These differences suggest the possibility that glutamatergic and GABAergic axons may have differential expression patterns of RPTP family members and splice forms (Takahashi and Craig).

Postsynaptic IL1RAcP and presynaptic PTPδ also function as a bidirectional excitatory synaptic organizing complex. IL1RAcP functions as a coreceptor with interleukin-1 receptor type I (IL1RI) for mediating immune and inflammatory responses to IL1 family cytokines, unlike IL1RAPL1 which lacks such function. IL1RAcP appears to serve two distinct functions, one with IL1 and IL1R1 in immune regulation and inflammation and another with PTPδ in synaptic adhesion and synapse organization (Takahashi and Craig, ; Yoshida et al.).

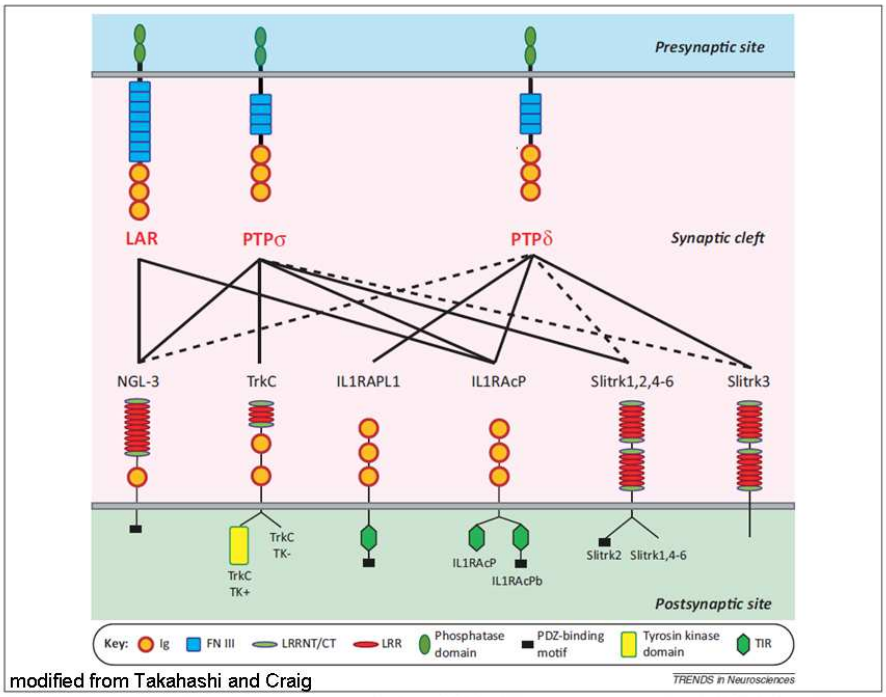


Fig. i. The selective binding code of receptor-type protein tyrosine phosphatases (RPTPs) with diverse postsynaptic partners: individual RPTPs bind to overlapping sets of postsynaptic partners, as indicated by the lines; broken lines indicate interactions that can occur *in vitro* but appear to lack physiological relevance. Importantly, except for netrin-G ligand-3 (NGL-3), these interactions are regulated by alternative splicing of RPTPs at the meA and meB sites (Takahashi and Craig).

The Slitrk family consists of six brain-specific transmembrane proteins (Slitrk 1–6) that are able to induce presynaptic differentiation. Intriguingly, Slitrk3 can induce inhibitory, but not excitatory, presynaptic differentiation. All Slitrks can interact with PTPδ (Takahashi et al.). RNAi-mediated knockdown of selective RPTPs in neurons co-cultured with Slitrk-expressing cells suggests that PTPs mediates the excitatory presynaptic differentiation induced by Slitrks, particularly Slitrk1 and Slitrk2 (Takahashi et al.). It is puzzling how Slitrk3–PTPδ can function selectively in inhibitory synaptic organization and IL1RAPL1/IL1RacP–PTPδ in excitatory synaptic organization. The PTPδ isoform that contains full meA and meB inserts, a major isoform of PTPδ in the brain, can bind to all Slitrks, IL1RAPL1, and IL1RacP, although perhaps with different affinity. Given that the meA and meB splice inserts of PTPδ differentially control its binding to IL1RAPL1 and IL1RAcP, it is possible that differential splicing of PTPδ in GABAergic versus glutamatergic axons contributes to selectivity in partner binding and function. Such a mechanism would be similar to the role of splicing in neurexins binding to different neuroligins. However, more complicated mechanisms involving axon-selective co-receptors or suppressors to regulate specificity of interactions cannot be ruled out. Whereas most of the other RPTP complexes have demonstrated bidirectional synaptic organizing activities, the Slitrk–PTPd/PTPs interaction has only been shown to induce presynaptic differentiation. A PTPd isoform that contains full meA and meB inserts and can bind to all Slitrks induces only excitatory not inhibitory postsyn-aptic protein clustering in co-culture assays, suggesting that Slitrk3 may need to cooperate with other synaptogenic complexes such as neurexin–neuroligin-2 for inhibitory synapse development (Takahashi and Craig).

Intracellularly, PTPδ interacts with MIM-B, a putative metastasis suppressor protein that is implicated in actin cytoskeletal control (Woodings et al., 2003). MIM was originally described as a protein whose mRNA was Missing in Metastasis. A variant of MIM, called MIM-B, was further characterized as a link between tyrosine kinase signalling and the actin cytoskeleton. MIM-B binds to the cytoplasmic domain of receptor PTPδ and the expression of full-length MIM-B induces actin-rich protrusions resembling microspikes and lamellipodia at the plasma membrane and promotes disassembly of actin stress fibres. The C-terminal portion of MIM-B is localized in the cytoplasm and does not affect the actin cytoskeleton when expressed, while the N-terminal portion localizes to internal vesicles and probably targets the protein to membranes. MIM-B may be a regulator of actin assembly downstream of tyrosine kinase signaling and this activity may explain the involvement of MIM in the metastasis of cancer cells (Woodings et al., 2003).

The interaction between IL1RAPL1 and PTPδ has been confirmed by a HEK293FT co-culture using IL1RAPL1 or IL1RAPL1ΔN (a deleted form of the protein, lacking the first and the second Ig-like domain) (Valnegri et al.). Only the full length form of IL1RAPL1 can enhance accumulation at sites of contact with PTPδ-transfected cells. The same experiment was performed with other members of LAR-RPTPs (LAR and PTPσ) and it excluded any interaction of these proteins with IL1RAPL1 (Valnegri et al.). Similarly, IL1RAPL2 specifically interacts with PTPδ but not with the other members of the LAR-RPTP family. Data have been confirmed by immunoprecipitation experiments (Valnegri et al.).

However, it has not been definitively shown whether dendritic recruitment of IL1RAPL1 by PTPδ is sufficient to recruit postsynaptic proteins, for example, by direct aggregation of IL1RAPL1 on dendrites this probability is supported by the finding that soluble IL1RAPL1 ectodomain inhibits postsynaptic differentiation by PTPδ (Yoshida et al.).

All these findings suggest that the IL1RAPL1complex, similarly to the neuroligin/neurexin complex, regulates trans-synaptic signaling that induces excitatory synapse and dendritic spine formation in brain.

Recently it has been discovered by affinity chromatography a new partner of IL1RAPL1: Mcf2-like (Mcf2l), a Rho guanine nucleotide exchange factor (GEF) that activates RhoA and Cdc42 and binds to TIR domain of IL1RAPL1. RhoA and Rac1 are implicated in the cytoskeletal dynamics that induce structural change of excitatory spines. Actin cytoskeletal dynamics are regulated by RhoA-dependent activation of ROCK (Rho-associated protein kinase) (Hayashi et al.).

Knockdown of endogenous Mcf2l and treatment with an inhibitor of ROCK suppressed IL1RAPL1-induced excitatory synapse formation of cortical neurons, suggesting that IL1RAPL1 controls spine formation of cortical neurons through Mcf2l-RhoA-ROCK signaling pathway. Because the suppression is not total it can be supposed that other pathway(s) besides Mcf2l-RhoA-ROCK one may support the endogenous IL1RAPL1-mediated spinogenesis when Mcf2l-RhoA-ROCK pathway in cortical neurons is blocked (Hayashi et al.).

Furthermore, it was found that the expression of IL1RAPL1 affected the turnover of AMPA receptor subunits and Mcf2l-RhoA-ROCK signaling pathway acts in the downstream of IL1RAPL1 in excitatory synapse stabilization (Hayashi et al., 2013). One process that is important in maintaining synaptic strength unchanged and in stabilization of recently formed synapses is the constitutive replacement of synaptic GluA1 by GluA2/3 (AMPAR subunits) (Granger et al.). This process takes up to 20 hr. The over-expression of IL1RAPL1 for 2 to 3 days in cortical neurons leads to the replacement of newly inserted AMPA receptor compositions through the Mcf2l-RhoA-

ROCK signaling pathway. In summary, IL1RAPL1, through Mcf2l-RhoA-ROCK signaling pathway, regulates the formation and stabilization of glutamatergic synapses between cortical neurons (Hayashi et al.).

All this data strongly suggests that most of the molecules found associated with ID regulate synapse formation and the consequential functional alteration or reduction in number of excitatory or inhibitory synapses arising from their mutations may alter the balance between excitatory and inhibitory synapses. This induces a general disorder within neuronal circuits and may be the direct cause of ID and ASD in humans. Indeed, even small changes in the expression and function of these proteins can provoke major alterations in synaptic connectivity, resulting in cognitive damages.

The learning deficiencies and memory declines observed in IL1RAPL1 knockout mice mimic the symptoms of ID children with *IL1RAPL1* mutations (Yasumura et al.). ID children with deletions in the *IL1RAPL1* gene have slow developmental milestones such as the onsets of walking and speech and require special education (Barone et al., ; Behnecke et al., ; Franek et al., ; Nawara et al., 2008). The *IL1RAPL1* gene is also associated with ASDs (Youngs et al., ; Bhat et al., 2008; Piton et al., 2008). ASDs are characterized by impairments in appropriate reciprocal social interactions, impairments in verbal social communication and high levels of ritualistic repetitive behaviours (Crawley, 2004). IL1RAPL1 knockout mice show a stereotyped behavior (Yasumura et al.) and their behavioural flexibility is slightly reduced in the reversal task of the T-maze left-right discrimination test but not in that of the Barnes maze test (Yasumura et al.). Interestingly, the performance of IL1RAPL1 knockout mice in the rotarod test is significantly better than that of wild-type mice (Yasumura et al.), which may be interpreted that IL1RAPL1 knockout mice prone to be stereotypic in behaviour since better performance in repetitive test of motor coordination was also reported for mutant mice exhibiting autistic behaviour (Kwon et al., 2006; Nakatani et al., 2009). However, the social interaction of IL1RAPL1 knockout mice results increased in one-chamber and Crawley's three-chamber social interaction tests (Yasumura et al.). In addition, vocal communications of IL1RAPL1 knockout mice are comparable to those of wild-type mice (Yasumura et al.).

Another characteristic feature of IL1RAPL1 knockout mice is an enhanced locomotor activity (Yasumura et al.), thus, hyperactive behaviour reported for ID patients with mutations in the *IL1RAPL1* gene (Barone et al., ; Behnecke et al., ; Franek et al., ; Nawara et al., 2008) is reproduced in IL1RAPL1 knockout mice (Yasumura et al.). In the social interaction test, hyperactivity may increase the number of contacts in IL1RAPL1 knockout mice, while the mean duration per contact was comparable between genotypes (Yasumura et al.). It is unlikely that locomotor activity would

strongly affect the indices employed in spatial and working memory tests (Yasumura et al.). Moreover IL1RAPL1 knockout mice show a the deficits in fear memories (Houbaert et al., ; Yasumura et al., ; Zhang et al.) and an anxiety reduction (Yasumura et al.).

Present studies with mutant mice revealed that the ablation of IL1RAPL1 affects diverse brain functions including learning, memory, behavioural flexibility, locomotor activity and anxiety (Pavlowsky et al., ; Yasumura et al., ; Zhang et al.).

Decrease of spine density in IL1RAPL1 mutant mice will cause excitation and inhibition imbalances in many brain circuits, since IL1RAPL1 is widely expressed in the brain (Houbaert et al., ; Zhang et al., ; Carrie et al., 1999). Thus, it is reasonable that multiple brain functions are affected by the mutation (Yasumura et al.).

For instance, the altered I/E balance at the hippocampo-BLA projections leads to a deficit in contextual memory expression rather than memory formation, thereby suggesting that cognitive disability in humans may result from the deficiency of synapses involved at different steps of the cognitive process, including memory restitution and behavioral expression. (Zhang et al.)

Human patients with *IL1RAPL1* mutations are classified as ID and/or ASDs (Behnecke et al., ; Franek et al., ; Youngs et al., ; Carrie et al., 1999; Tabolacci et al., 2006; Bhat et al., 2008; Nawara et al., 2008; Piton et al., 2008). It remains to be examined how neural circuits responsible for these mental disorders are mainly affected by *IL1RAPL1* mutations (Yasumura et al.).

MUTATIONS OF *IL1RAPL1*

Different mutations in *IL1RAPL1* gene have been discovered in members of families with intellectual disability and autism.

First, Carrièt et al. identified a patient with a C-A transition in exon 11 that changes the codon codifing tyrosine (TAC) in a stop codon (TAA) (Y459X): it results in a protein lacking a part of the TIR domain and all the C-terminal domain (Carrie et al., 1999).

In another family, three male with ID and IQ<70 have a truncated form of protein that lacks the last 210 aminoacids in the cytoplasmic domain, because of a transition G1460A in exon 10 of gene resulting in a substitution trp487stop (W487X) (Tabolacci et al., 2006). Moreover in three brothers with IG 55, hyperactivity, attention deficit, auto-aggressivity, dysmorphism, has been discovered a truncated form of IL1RAPL1 due to a deletion in exon 11 (Youngs et al.).

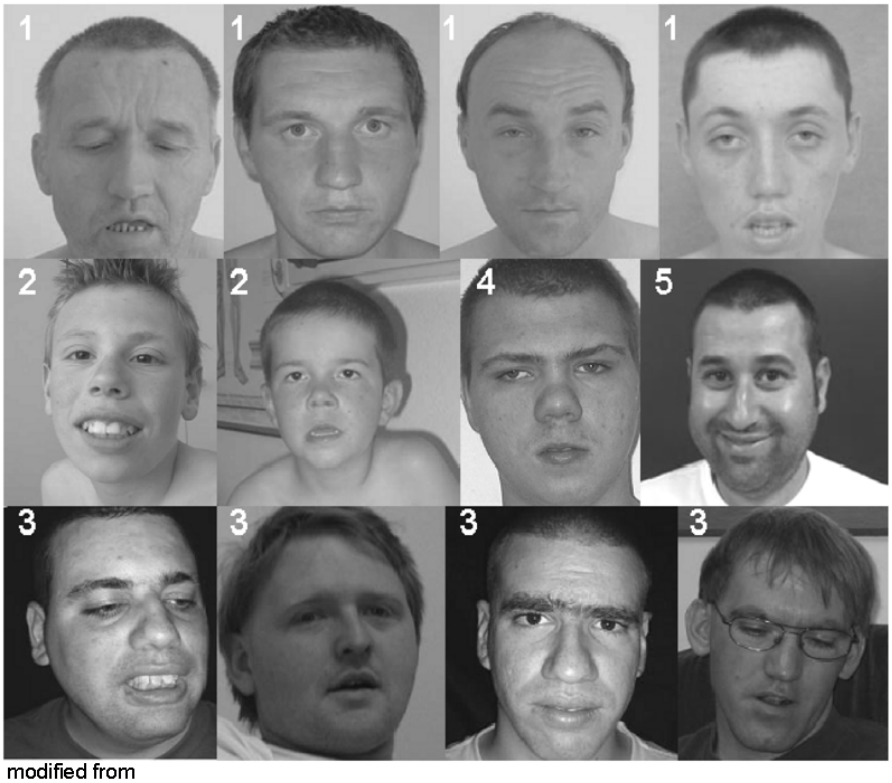


Fig. l. Patients with mutations or deletions in *IL1RAPL1* gene show some facial features like strabism, ptosis, high nasal bridge and broad nasal base, large mouth and macrodontia of the central maxillary incisors, etc.

Another mutation (seven nucleotide deletion c.1730delTACTCTT) in exon 9 cause a frameshift at Ile367 with a premature stop codon (TGA) 6 codons downstream (p.Ile367SerfsX6) resulting in a truncated protein that lacks a part of the transmembrane domain as well as the entire cytoplasmic domain: the protein can't reach the cell surface (Piton et al., 2008). In another family, a frameshift mutation (A28EfsX15) results in the exclusion of exons 3, 4, 5, 6 and 7 (Piton et al., 2008). Last two mutations described are involved both in ID and ASD (Piton et al., 2008). An other deletion in the extracellular domain (exons 2, 3, 4, 5 and 6 of the gene) was described by Nawara et al., 2008 and Franek at al., 2011 (Franek et al., ; Nawara et al., 2008). Patients present hyperactivity ID, aggressivity (Nawara et al., 2008), low IQ and dysmorphism (Franek et al.).

The same research group discovered in two male brothers a deletion in exons 1–5; in this case IL1RAPL1 protein is absent in patients and, interestingly, they don't correlate with ASD (Franek et al.). Deletions of exons coding for the extracellular regions has been found in two German patients with low IQ that belong to two different-non related families and share psychomotor development delay: one has exon 2 deleted, in the other one the deletion starts 245454 bp after exon 2 and ends 117424 bp after exon 5 (Behnecke et al.). An Italian patient with ID, ASD and an epilepsy episode presents a 285 Kb deletion in chromosome Xp21.3-21.2, with breakpoints lying in *IL1RAPL1* gene exon 3 (Barone et al.).

It has also been identified an inversion in chromosome X, inv(X)(p21.3q27.1) (Lepretre et al., 2003) and a complex deletion-inversion that drives to a loss of different gene and to a creation of a fusion protein between IL1RAPL1 and distrofin (Wheway et al., 2003).

Interestingly also a duplication can lead to pathology: a 15.5-Mb duplication (Xp11.4-p21.3 region containing 41 genes including IDX *IL1RAPL1* and *TSPAN7*) can leads to ID, suggesting the importance of regular dosage of protein (Froyen et al., 2007).

During my phD in Carlo Sala laboratory, we decided to study the function of three novel independent mutations on IL1RAPL1 in patients presenting mild to moderate ID: IL1RAPL1Δexon6 (Ramos-Brossier et al.), IL1RAPL1 C31R (Ramos-Brossier et al., ; Tarpey et al., 2009) and IL1RAPL1 I643V (Ramos-Brossier et al., ; Piton et al., 2008). Two of them affecting the same exon, and unlike most of previously reported mutations, those are predicted to lead to protein synthesis but to damage extracellular domain. The identification of these novel mutations on *IL1RAPL1* and the clinical characterization of the patients is reported in (Ramos-Brossier et al.).

Most of the mutations and deletions involving *IL1RAPL1* gene are summarized in table m.

The majority of the described mutations include large deletions, and it is of particular interest that they mostly involve the first exons coding for extracellular domain of IL1RAPL1 protein. Some authors suggest that because of the incidence of genomic rearrangements, such as pericentromeric

inversions, this region must be particularly prone to recombination (Franek et al., ; Lepretre et al., 2003; Nawara et al., 2008).

In summary, most of mutations described above leads to the absence of IL1RAPL1 production or to a small or truncated form of the protein: this can result in the loss of some protein-protein interactions due to the lack of interacting regions. Alternatively, other mutations result in a completely loss of the protein or loss of the correct localization (Barone et al., ; Behnecke et al., ; Franek et al., ; Koh et al., ; Youngs et al., ; Carrie et al., 1999; Bahi et al., 2003; Lepretre et al., 2003; Wheway et al., 2003; Tabolacci et al., 2006; Froyen et al., 2007; Nawara et al., 2008; Piton et al., 2008).

Interestingly, mutations and deletion of *IL1RAPL1* gene are related to different phenotypes (even in the same family and in patients with the same mutation) including different severity of ID, association or not with ASD and other physical features. Moreover, the phenotype of carrier female is interesting: even if most of the carrier females have normal cognitive skills, there are some cases of mild cognitive impairments or learning difficulties, maybe due to a different X-inactivation pattern (Tabolacci et al., 2006).

Table m. Reported mutations on *IL1RAPL1* gene in ID patients, and their consequences for protein function.

References	Mutation/deletion	Domains involved in the mutation/deletion	Protein	Phenotype	Functional consequences
Ramos-Brossier, Montani et al., 2014 Tarpey et al., 2009	missense exon 3	N-term	C31R	IQ 36-51, gynecomastia, obesity, small testes, sexual deviant behavior, significant behavioral issues, no phenotype in female	No effect on spines number, less interaction with PTPδ, No effect on excitatory synapses formation
Ramos-Brossier, Montani et al., 2014 Piton et al., 2008	missense exon 11	C-term	I643V (Full lenght)	ID	Same effects as the full-lenght protein
Ramos-Brossier, Montani et al., 2014	deletion exon 6	N-term	Shorter extracellular domain, protein instability (Δex6)	ID, behavioural problems, pseudo autistic behaviour, language and motor delay, dysmorphism, generalized joint hyperlaxity, skin significant for eczema	No effect on spines number, less interaction with PTPδ, No effect on excitatory synapses formation, Uncorrect localization
Redin et al., 2014	deletion exon 7		Truncated protein containig only the first two Ig-like domains		
Mignon-Ravix et al., 2014	deletion exons 3-5	N-term	Frame deletion of 207 amino acids (N29_A235del)	ID, motor and language delay, dysmorphism	
Tucker et al., 2014	deletion exon 3	N-term	Probably not produced	/	
Koh et al., 2013	Xp21 contiguous gene deletion syndrome		Probably not produced	X-linked adrenal hypoplasia congenita, central precocious puberty, ID	
Barone et al., 2013	deletion exon 3	N-term	Probably not produced	IQ 41, attention deficit, dysmorphism	
Youngs et al., 2012	deletion exons 3-11	N-term and C-term	Probably not produced	IQ 55, hyperactive, attention deficit, autoaggressive, dysmorphism	
Behnecke et al., 2011	deletion exon 2	N-term	Probably not produced	IQ 70–85, hyperactive, dysmorphism	
Behnecke et al., 2011	deletion exons 3-5	N-term	w/o 2 Ig-like domains (ΔN) or not produced	IQ 44–60, learning difficulties, attention deficit, dysmorphism in male	No effect on spines number, No interaction with RhoGAP2 and PTPδ, No effect on excitatory synapses formation
Franek et al., 2011	deletion exons 1-5	N-term	Probably not produced	depression, dysmorphism	
Franek et al., 2011	deletions exons 3-6	N-term	w/o 2 Ig-like domains (ΔN)	IQ 41–66, impulsive, oppositional, hyperactive, dysmorphism	No effect on spines number, No interaction with RhoGAP2 and PTPδ, No effect on excitatory synapses formation
Mikhail et al., 2011	deletion exons 2-6	N-term	Probably not produced	ID, pervasive developmental disorder, language delay, dysmorphism, diminished tone and strenght	
Whibley et al., 2010	deletion exons 3-5	N-term	Probably not produced	moderate ID	
Nawara et al., 2008	deletion exons 3-6	N-term	Probably not produced (ΔIg123)	ID in male, hyperactive, autoaggressive	Uncorrect localization, No effect on excitatory synapses formation
Piton et al., 2008	deletion exons 3-7	N-term	A28EfsX15 (Signal peptide+8aa)	ID in male, autism, attention deficit hyperactivity, pervasive developmental disorder	
Piton et al., 2008	7 nucleotide deletion in exon 9	Transmembrane domain	I367SfsX6 (w/o half of the TIR and the entire C-ter domains)	ID in male, autism, attention deficit hyperactivity, pervasive developmental disorder	Uncorrect localization, cotransf of miRNA vs IL1RAPL1+I367SfsX6 results in high neurite number and length (like miRNA alone)
Tabolacci et al., 2006	nonsense in exon 10	C-term	W487X (ΔC)	IQ<70	No effect on spines number, No interaction with RhoGAP2, Increased excitatory synapses formation
Carrie et al., 1999	nonsense in exon 11	C-term	Y459X (ΔC)	IQ<70	No effect on spines number, No interaction with RhoGAP2, Increased excitatory synapses formation
Carrie et al., 1999	deletion exons 3-5	N-term	Probably not produced (ΔN)	IQ<70	No effect on spines number, No interaction with RhoGAP2 and PTPδ, No effect on excitatory synapses formation
Kozak et al., 1993	nonsense in exon 11	C-term	W487X (ΔC)	delayed psychomotor development, ID, dysmorphism	No effect on spines number, No interaction with RhoGAP2, Increased excitatory synapses formation

AIM OF THE WORK

Interleukin-1 receptor accessory protein like 1 (*IL1RAPL1*) gene is localized on X chromosome and mutations/deltions of this gene are strongly associated to ID and autism spectrum disorder (ASD) (Carrie et al., 1999; Piton et al., 2008). IL1RAPL1 is localized at the postsynaptic compartment of excitatory synapses (Pavlowsky et al.) and plays an important role in synapse formation and stabilization in developmental brain (Pavlowsky et al., ; Valnegri et al.).

Several studies indicate that ID is associated with abnormalities of dendrites and dendritic spines, resulting in a defect in neuronal connectivity and thus an altered processing of information at both the cellular and the neural network. Thus, functional analysis of IL1RAPL1 might be of interest to identify the molecular or cellular mechanisms that contribute to cognitive functions.

Even if IL1RAPL1 function has been already described (Hayashi et al., ; Houbaert et al., ; Pavlowsky et al., ; Valnegri et al., ; Yasumura et al., ; Yoshida et al., ; Gambino et al., 2007; Yoshida and Mishina, 2008; Gambino et al., 2009), there is an incomplete view of which effects of the mutations or the absence of the protein have on synapses, neurons, and neural networks. Accordingly, the aim of presented work was to characterize IL1RAPL1 mutants identified in patients with intellectual disability and autism (this part of the data has already been published (Ramos-Brossier et al.), thus the results and their discussion are not fully reported in this Thesis) and to perform a behavioural and neuronal morphology analysis on IL1RAPL1 KO mice. In particular, we decided to study the function of three novel independent mutations on IL1RAPL1 in patients presenting mild to moderate ID: IL1RAPL1Δexon6, IL1RAPL1 C31R and IL1RAPL1 I643V (Ramos-Brossier et al.). Two of them affecting the same exon, and unlike most of previously reported mutations, those are predicted to allow protein synthesis of a mutated protein with a damaged extracellular domain (Ramos-Brossier et al.).

In particular, through over-expression of the IL1RAPL1 mutants in rat primary cultured neurons, we studied their localization, the ability to recruit presynaptic compartment and to induce changes in neuronal morphology and spine density. To better understand the molecular interaction between IL1RAPL1 and PTPδ, we also investigate, through a cell aggregation assay, the residual ability of IL1RAPL1 mutants to bind PTPδ (Ramos-Brossier et al.). Moreover, given that dendritic abnormalities are one of the most consistent anatomical correlates of ID, we also characterize the role of wild type and mutants IL1RAPL1 in regulating dendrite morphology using both *in vitro* neuronal culture and IL1RAPL1 KO mice.

Furthermore, IL1RAPL1 WT and KO mice have been analyzed with behavioural test to point out differences in motor activity, memory and anxiety.

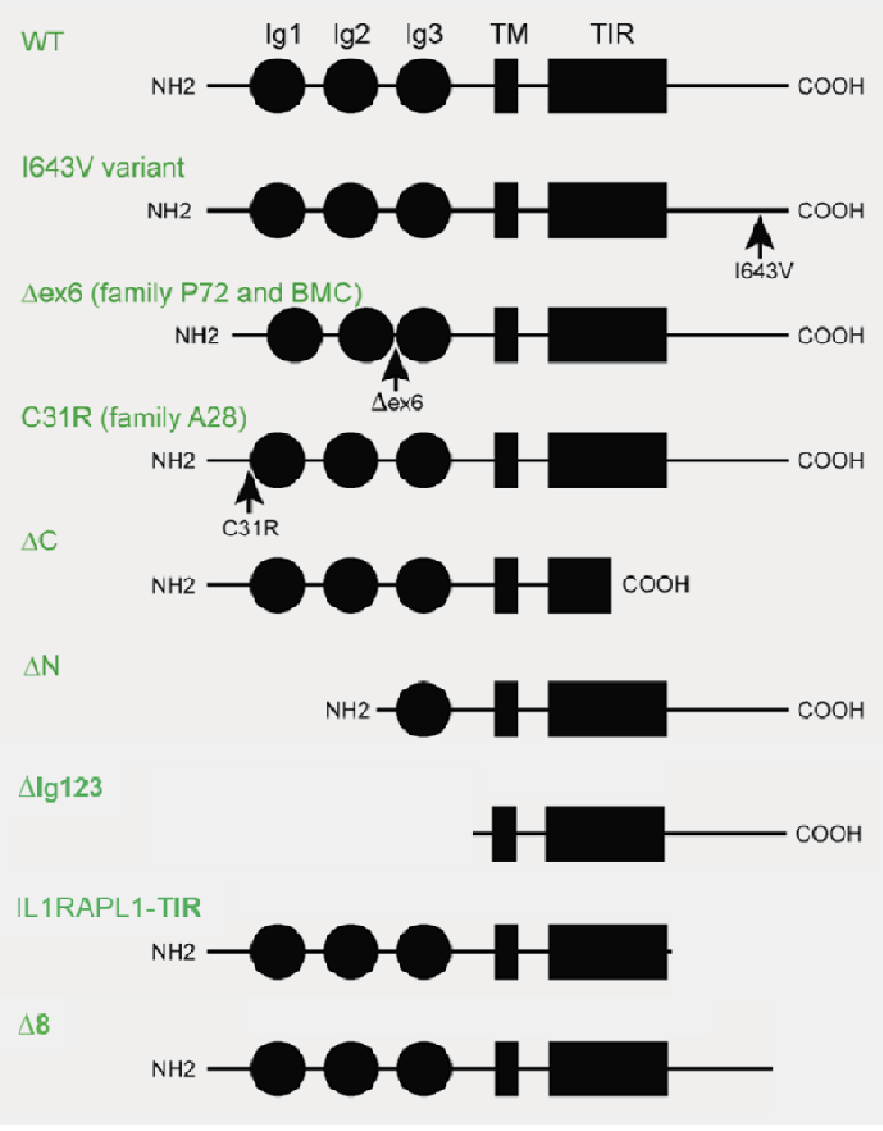
Understanding how mutations or absence of IL1RAPL1 act on synaptogenesis and dendritic morphology can help to clarify how any changes in IL1RAPL1 function can lead to cognitive disorders in humans.

In addition, further investigation of the molecular mechanism of IL1RAPL1 would identify potential drug targets and IL1RAPL1 knockout mice will be useful to assess the new possible treatments.

MATERIALS AND METHODS

cDNA CONSTRUCTS

HA-tagged human IL1RAPL1 described before (Pavlowsky et al.) was modified using QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) to generate Δex6, C31R, I643V and ΔIg123 constructs (Ramos-Brossier et al.). Myctagged PTPδ, IL1RAPL1 ΔC, IL1RAPL1 ΔN, IL1RAPL1 Δ8 were described elsewhere (Pavlowsky et al., ; Valnegri et al.).



modified from Ramos-Brossier et al

Human PTPδ full length is a gift from E. Kim (Kwon et al.). Rat PTPδ without miniexons A and B is a gift from M. Mishina (Yoshida et al.). shRNA plasmid against rat PTPδ is a gift from Ann Marie Craig.

All this transmembran proteins cDNA is inserted in GW1-CMV vector. The signal peptide of IL1RAPL1 (aa 1-25) and PTPδ was switched with signal peptide of GluR2 (AMPA receptor

subunit), a not cleavable signal peptide to ensure a cytoplasmic membrane localization and, downstream, HA tag was inserted. As control, pEGFP vector was also used.

ANTIBODIES

The following primary antibodies were used: rabbit anti-IL1RAPL1 (K10, (Pavlowsky et al.)), goat anti-IL1RAPL1 (R&D), rabbit anti-IL1RAPL1 (Proteintech), mouse anti-GFP (Roche and Abcam), rabbit anti-VGLUT1 (Synaptic Systems), rabbit anti-VGAT (Synaptic Systems), rabbit anti-HA-tag (Santa Cruz Biotechnology), mouse anti-HA-tag (Roche), mouse anti-c-Myc (Santa Cruz Biotechnology), mouse anti-PSD-95 (Affinity Bioreagents), rabbit anti-synaptophysin (Cell Signaling and Sigma Aldrich), mouse anti-GAPDH (Ambion), mouse anti-LAR (Neuromab), rabbit anti-PTPδ (Santa Cruz Biotechnology), goat anti-ARHGAP22/RhoGAP2 (Santa Cruz Biotechnology), rabbit anti-mGluR5 (Millipore), mouse anti-mGluR1/5 (Neuromab), rabbit anti-GluR1 (Millipore), mouse anti-GluR2 (Neuromab), mouse anti-GluR2/3 (gift from Cecilia Gotti), mouse anti-NR1 (Neuromab), mouse anti-NR2B (Neuromab), rabbit anti-ERK 1/2 (Cell Signaling), mouse anti-panShank (Neuromab), rabbit anti-Shank3 (Santa Cruz Biotechnology), mouse anti-Homer (Santa Cruz Biotechnology), mouse anti-PSD-95 (Neuromab), mouse anti-Neuroligin 1 (gift from Nils Brose), mouse anti-βActin (Sigma Aldrich), mouse anti-GAD-65 (Abcam).
All HRP-conjugated secondary antibodies were purchased from Jackson ImmunoResearch Labs.
All fluorophore-conjugated secondary antibodies were purchased from Jackson ImmunoResearch Labs.

CELL CULTURE AND TRANSFECTION OF PRIMARY RAT AND MOUSE HIPPOCAMPAL NEURONS

Low-density rat hippocampal neuronal cultures were prepared from embryonic day (E) 18-19 hippocampi as previously described with minor modifications (Verpelli et al., ; Sala et al., 2001) and were grown in 12-well Petri dishes (Primo).
Neurons were transfected using Lipofectamine 2000 on Days In Vitro 11 (DIV11) and experiments were performed at DIV14-18. Experimental procedures on animals were approved by the local ethical committee. At DIV1 Neuronal primary cultures were transfected with calcium chloride method as described previously (Verpelli et al.) and experiments were performed at DIV4.

IMMUNOCYTOCHEMISTRY

Cells were fixed in 4% PFA plus 4% sucrose at room temperature for 20 minutes, or 100% methanol at -20° for 10 min. Primary (1:100-1:800) and secondary (1:200) antibodies were applied

in GDB buffer (30 mM phosphate buffer, pH 7.4, 0.2% gelatin, 0.5% Triton X-100, 0.8 M NaCl (all Sigma-Aldrich)) or in PBS (136.8 mM NaCl, 2.68 mM KCl, 10.1 mM Na_2HPO_4 and 1.76 mM KH_2PO_4 , pH 7.4 (all Sigma-Aldrich)) containing 3% BSA and 0.2% Tween 20. Primary antibodies were applied for 1-3 h at room temperature. Secondary antibodies conjugate with fluorophores (Jackson Immunoreserch) were also diluted in GDB buffer and applied for 1 h. After each antibody incubation, 3 washes (10 min each) took place with "high-salt buffer" (20mM Na2HPO4/NaH2PO4 and 0.5M NaCl, pH 7.4 (all Sigma-Aldrich)) and before mounting a final wash (for 10 min) was carried out with PBS. Before mounting, coverslips were briefly passed through ddH_2O to dilute salts. 4',6-diamidino-2-phenylindole (DAPI) staining (Life Technologies) was carried out for 2 min (DAPI diluted in PBS to a final concentration of 0.5 µg/ml) and took place during the washing steps before mounting the coverslips with Mounting Medium (Vecta Shield).

NEURON SURFACE STAINING

At DIV 14-15, live hippocampal neurons were labeled for 10 min at 37°C with anti-HA-tag rabbit antibody (10 µg/ml). After washing, neurons were fixed with paraformaldehyde (PFA) 4% plus 4% sucrose and incubated with anti-HA-tag mouse antibody in GDB (30 mM phosphate buffer, pH 7.4, 0.2% gelatin, 0.5% Triton X-100, 0.8 M NaCl (all Sigma-Aldrich)) for 3 h at room temperature. Cells were washed in 20 mM phosphate buffer containing 0.5 M NaCl and incubated with FITC- and Cy3-conjugated secondary antibodies.

IMAGE ACQUISITION AND PROCESSING

Confocal images were obtained using a Zeiss 510 confocal microscope (Carl Zeiss, a gift from Fondazione Monzino) or a Leica DMI6000 Spinning disk microscope (PerkinElmer, a gift from Fondazione Monzino) with Zeiss 63 x, 40x or 20x objectives at a resolution of 1024 x 1024 pixels. Images represent maximum intensity projections of five individual images taken at depth intervals of around 0.5 µm.

Quantification of synaptic protein staining was performed using MetaMorph (Molecular Devices, Downingtown, PA), and ImageJ software and NeuronJ plugin (Meijering et al., 2004). Labeled, transfected cells were chosen randomly for quantification from six coverslips from 3 independent experiments for each condition and image analysis was performed under blind condition.

Quantification of protein surface staining was performed using MetaMorph (Molecular Devices, Downingtown, PA), and ImageJ software. The ratio of integrated intensity of surface rabbit anti-HA signal per total mouse anti-HA signal was measured for each neuron. Then we calculated the mean and SEM for the neurons transfected with the same construct.

Sholl analysis was performed using NeuronStudio (Computational Neurobiology and Imaging Center Mountain Sinai School of Medicine, New York, NY). Sholl analysis is a method of quantitative analysis used in neuronal studies to characterize the morphological features of an imaged neuron. It creates a series of concentric circles around the soma of the neuron. Within each sphere various metrics can be obtained such as the total length of intersecting dendrites or the number of branching points. We performed Sholl analysis to measure the number of branching points in order to evaluate the dendritic arborization complexity in adult neurons (DIV14-15).
Young neurons (at DIV4) were analyzed manually: the number of primary and secondary dendrites was counted together with the total length of counted dendrites using MetaMorph softwere.

DATA ANALYSIS AND FIGURE DISPLAY

Data was analyzed for statistical significance and displayed by Prism 5 software (GraphPad, San Diego, CA). If there were only two groups whose means were compared, a student's t-test was carried out to assess statistical significance. The accepted level of significance was $p \leq 0.05$. To compare more than two groups, one factorial analysis of variance (ANOVA) was used and if group means differed in a significant manner ($p \leq 0.05$), a post hoc Dunnet (groups compared to the control) and Tukey (all groups compared) test was calculated to assess statistical significance. The accepted level of significance for the post hoc test was $p \leq 0.05$.
In graph, data are presented as mean ± SEM.

CULTURED NEURONS OR BRAIN LYSATES IMMUNOBLOT ANALYSIS

Cultured neurons or brain lysates were collected with precooled "sucrose buffer" (0.32 M sucrose (Sigma-Aldrich)/4mM HEPES-NaOH buffer (Sigma-Aldrich), pH 7.3, protease inhibitors (Roche), phosphatase inhibitors (Roche)) and analyzed via Bradford protein assay (Bio-Rad) to asses protein concentration. For total lysate (TL), proteins were solubilized in 2-4x loading buffer/dye ((250 mM Tris, 8 % (w/v), 40 % (v/v) glycerol, 0.008 % (w/v) bromophenol blue (all Sigma-Aldrich)) in order to have a final protein concentration of 1 µg/µl in the sample. In other cases, fractionation took place prior to Bradford protein assay analysis resulting in a P1 fraction (enriched in cell bodies and dendritic fragments) and a P2 or crude synaptosomal fraction (enriched in presynaptic and postsynaptic components) (Grabrucker et al., ; Huttner et al., 1983). Also in the case of P1 and P2 fractions, 2-4x loading buffer was added in order to have a final protein concentration of 1 µg/µl in the sample. 1mM DTT (DL-Dithiothreitol, Sigma-Aldrich) was added to the samples for reducing protein disulfide bonds. Samples were then heated for 10 min at 65°C (thermoblock from FALC Instruments) and 25 mM Iodoacetamide (Sigma-Aldrich), an alkylating reagent for cysteine

residues was added to eliminate artifacts of disulfide formation during electrophoresis. 10 µg of samples were loaded in the pockets of 6-15% polyacrylamide gels (home made with reagents from Bio-Rad) and proteins were electrophoretically seperated by sodium dodecyl sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were electrophoretically seperated by SDS-PAGE under denaturing conditions (Laemmli, 1970) followed by electroblotting of the proteins onto a nitrocellulose membrane using the Trans-Blot Turbo System (Bio-Rad). Then the membranes were stained by Ponceau S Stain (Sigma-Aldrich) to control for efficient protein transfer of proteins and were subsequently washed twice in Tris-buffered saline-Tween (TBS-T) (20 mM Tris pH 7.4, 150 mM NaCl (both Sigma-Aldrich), and 0.1% Tween 20 (Bio-Rad)).
Blocking of membranes took place for at least 1.5 h at 4°C in blocking buffer (TBS-T and 5% dried nonfat milk). Primary antibodies were applied for 1-3 h in blocking buffer (here: with 3 instead of 5% dried nonfat milk). For rat neuronal culture lysates, HRP-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) were used in blocking buffer (here: with 3 instead of 5% dried nonfat milk) and chemiluminescence was induced using an ECL kit (GE Healthcare).
Normalization took place via actin for both TL and P2 samples.

ANIMALS

To prepare primary neuronal rat cultures, pregnant female rats (Rattus norvegicus) of the phylum Wistar were purchased from Charles River (Charles River Laboratories, Calco, Italy).
IL1RAPL1-KO mice are derived via Cre-LoxP by substitution of exon 5 with a stop codon on a C57BL/6 background (Charles River Laboratories, Calco, Italy) as described before (Takagi et al., 2002; Gambino et al., 2009).
Mice and rats were housed under constant temperature (22 ± 1°C) and humidity (50%) conditions with a 12 h light/dark cycle, and were provided with food and water ad libitum. For proteomic analysis, Golgi staining and behavioral tests of IL1RAPL1-KO and WT mice, 3 months old littermates were used.
All experiments involving animals followed protocols in accordance with the guidelines established by the European Communities Council and the Italian Ministry of Health (Rome, Italy). Experimental procedures of behavioral analysis followed the guidelines established by the Italian Council on Animal Care and were approved by the Italian Government decree No. 17/2013. All efforts were made to minimize the number of subjects used and their suffering.

GENOTYPING OF MICE

For genotyping of mice, DNA was extracted from tails and analyzed by PCR with the REDExtract-N-Amp^{TM} Tissue PCR Kit (Sigma-Aldrich).

GOLGI STAINING

Mice were perfused transcardially with 0,9% NaCl in distilled water, the brain was taken and immersed in Golgi-Cox solution (1% $K_2Cr_2O_7$, 1%$HgCl_2$, 1% K_2CrO_4 (all Sigma-Aldrich)) (Glaser and Van der Loos, 1981) for 2-6 days in the dark, at room temperature. Golgi-Cox solution should be prepared at least five days before the impregnation, stored in the dark and filtered before use.

After the period of impregnation, the brain was washed three times with distilled water, immersed in a 30% sucrose solution and stored at 4°C, in the dark, for at least one week.

The brain was cutted with a vibratome setting frequency at 5.0, speed at 3.0 and slice thickening at 80-100 μm; during the operation the sample was kept wet with a 6% sucrose solution. Slices were put on gelatinized slides (2% gelatin, 1% $Kcr(SO_4)12H_2O$).

Slices was stained in the dark: first, slides were washed 2 times with distilled water for 5 minutes, then put 30 minutes in ammonium hydroxide (Sigma-Aldrich). After another step of distilled water washes, slides were put in Kodak solution (Sigma Aldrich) for 30 minutes. Slides were washed 2 times in distilled water, and then 1 minute in each crescent percentage of ethanol (Fluka): 50%, 70%, 95% and 2 times for 5 minutes in 100% ethanol. Finally slides were put for 15 minutes in solution X (1/3 chloroform (Sigma-Aldrich), 1/3 xylene (Carlo Erba), 1/3 ethanol) and then 15 minutes in xylene. Slides were mounted on coverslips with Entellam (Electron Microscpy Sciences). Images were with 20x objective in white field. Neurons were analyzed with Neuron Studio software to perform a Sholl analysis of branching points.

OLFACTORY TEST

Two days before the test, an unfamiliar palatable food (Kellogg's chocolate cereal) is placed overnight in the home cage of the subject mice. On the day of the test, each mouse is placed in a large cage containing 3 cm deep sawdust and allowed to explore for 5 minutes. The animal is removed from the cage and one cereal is buried in the cage bedding. The animal is then returned to the cage and given 15 minutes to locate the buried food. Latency to find the cereal is recorded.

SPONTANEOUS MOTOR ACTIVITY

Spontaneous motor activity was evaluated as previously described (Braida and Sala, 2000) in an automated activity cage (43×43×32 cm) (Ugo Basile, Varese, Italy), placed in a sound-attenuating

room. The cage was fitted with two parallel horizontal and vertical infrared beams located 2.5 cm and 5 cm from the floor, respectively.
Cumulative horizontal and vertical beam breaks were counted for 60 min.

ELEVATED PLUS MAZE

The Elevated Plus Maze paradigm was used to study anxiety related behavior. The apparatus consisted of two opposite open arms (30 cm x 10 cm) and two enclosed arms (30 cm x 10 cm x 14 cm) that extended from a common central platform (10 cm x 10 cm) according to Lister (Lister, 1987). The apparatus was built with white wood, elevated to a height of 60 cm above floor level and placed in the center of a small quiet room under dim light (about 30 lux). Testing was conducted during the early light phase (9.30–13.30 am) of the light cycle. After 20 min adaptation to the novel surroundings, mice were placed individually onto the center of the apparatus facing an open arm. The number of open- and closed-arm entries and the time spent in open and closed arms were recorded for 5 min.

MORRIS WATER MAZE

The water maze was a circular tank (diameter 1.5 m) filled with water (25°± 0.5°C). A platform (13.5 cm x 23 cm) was submerged below the water's surface in the center of the target zone. Floating polystyrene particles were placed on the surface of the water to hide the platform from sight. Extra maze cues (simple geometrical shapes) and visual references (chair, table) were present around the room to provide spatially oriented cues. Intra-maze cues along the edge of the pool were also located. Four points around the circumference of the maze were arbitrarily designated as N, S, E, and W, which served as a reference for experimenters when releasing the mice into the pool. Within each acquisition session (i.e. 4 trials in a day), mice were randomly released from each of the four points, facing the pool wall.
After one day of habituation in the maze for 60 s without the platform, acquisition training consisted of 4 trials per day for four consecutive days and an intertrial interval of 1 h. Once the mouse located the hidden platform, the mouse remained on the platform for 30 s before removal from the tank. If a mouse failed to locate the platform within 120 s, it was guided to the platform and remained there for 30 s before removal from the pool. The sixth day, the platform was removed (probe test) and the animals were allowed to remain in the pool for 120 s. An experimenter, blind to the genotype, manually recorded the escape latency and time spent in the target zone of the maze.

PASSIVE AVOIDANCE

Briefly, the apparatus consisted of two compartments, one light and one dark, connected via a sliding door. In the acquisition trial, each mouse was placed in the light compartment and allowed to enter the dark compartment; the time (in s) taken to do so was recorded. Mice having latencies greater than 40 s were eliminated. Once the mouse was in the dark compartment, the sliding door was closed and an unavoid- able electric shock (1 mA for 1 s) delivered via the paws.

The animal was then placed back in the home cage until the retention trial. The retention trial was carried out 30 min or 24 h after the acquisition trial, by positioning the mouse in the light compartment and recording the time taken to enter the dark compartment (retention latency). An increased retention latency indicates that the animal has learned the association between the shock and the dark compartment. During the retention trial, a cut-off time of 180 s was used.

RESULTS

NOVEL *IL1RAPL1* MUTATIONS ASSOCIATED WITH INTELLECTUAL DISABILITY IMPAIR SYNAPTOGENESIS

We studied the function of three novel independent mutations of IL1RAPL1 gene in patients presenting mild to moderate ID (Ramos-Brossier et al.): IL1RAPL1Δexon6, IL1RAPL1 C31R and IL1RAPL1 I643V. Through over-expression of the IL1RAPL1 mutants in rat primary cultured neurons, we studied their localization, the ability to recruit presynaptic compartment and to induce changes in neuronal morphology and spine density. To better understand the molecular interaction between IL1RAPL1 and PTPδ, we also investigate, through a cell aggregation assay, the residual ability of IL1RAPL1 mutants to bind PTPδ (Ramos-Brossier et al.).

We found that two of the studied mutants lead to a partial loss of function of IL1RAPL1 protein, which is, first, responsible for the cognitive impairments observed in patients and, second, highlights the important function of the extracellular domain for the trans-synaptic PTPδ/IL1RAPL1 interaction in synaptogenesis.

This part of the data has already been published (Ramos-Brossier et al.), thus the results and their discussion are not fully reported in this Thesis.

THE X-LINKED INTELLECTUAL DISABILITY PROTEIN IL1RAPL1 REGULATES DENDRITIC ARBORIZATION

Dendritic arborization complexity of hippocampal neurons is altered in IL1RAPL1-KO adult mice

Because genetic form of intellectual disability and autism are often associated to alteration in dendrites morphology, we decided to analyse dendritic arborisation complexity in wild type and IL1RAPL1 KO mice using Golgi staining of CA1 (Fig. 1), CA2 (Fig. 2) and Occipital and Parietal cortex neurons (Fig. 3) from 3 months old male littermates,. Sholl analysis was performed on 8 neurons for each brain field for 4-9 animals of each genotype using Neurostudio softwere. Total number of branching points was counted (Fig. 1b, 2b, 3b) and plotted on the distance from the soma (Fig. 1c, 2c, 3c).

We observed an increased number of branching points in IL1RAPL1-KO mice hippocampal neurons of CA1 and CA2 fields. On the contrary, the number of branching points of cortical neurons from WT and KO mice is comparable.
We can conclude that the absence of IL1RAPL1 *in vivo* affects the correct dendrite development in the hippocampus.

Identification of IL1RAPL1 protein domains involved in regulating dendritic arborization complexity in rat neuronal primary colture

Hippocampal neurons were transfected at DIV11 with GFP, HA-IL1RAPL1, HA-IL1RAPL2, HA-IL1RAPL1ΔN, HA-IL1RAPL1Δexon6, HA-IL1RAPL1 C31R, HA-IL1RAPL1ΔC, HA-IL1RAPL1Δ8, HA-IL1RAPL1 TIR or HA-IL1RAPL1 I643V and were fixed and stained at DIV14-15 with anti HA and anti MAP2 antibodies to label dendrite morphology (Caceres et al., 1984) (Fig. 4). Images obtained by confocal microscopy (Fig.4a) were subjected to Sholl analysis and the total number of branching points was quantified (Fig. 4b) and plotted on the distance from the soma (Fig. 4c).
We found that the overexpression of IL1RAPL1 induce a reduction in number of branching points compared to the control (GFP expressing neurons). The overexpression of constructs that present mutations and deletions in the intracellular domains of IL1RAPL1 leads to a decreased number of branching points like the full length does. On the contrary, the overexpression of mutants in the extracellular domains seems not to have an effect on arborization or induce a higher dendrite complexity (IL1RAPL1 C31R overexpression) compared to the control.

We can conclude that the extracellular part of IL1RAPL1 protein seems to be involved in controlling dendrite arborization.
Given that the only know interactor of IL1RAPL1 extracellular domain is PTPδ, we decided to understand wether this *trans*-interaction could be involved on the effect on dendrite arborization.
In vitro, synaptogenesis process starts later than DIV 4 (Baj et al.), thus we decided to use immature neurons to exclude the role of *trans*-synaptic interaction between IL1RAPL1 and PTPδ in the IL1RAPL1 mediated effect on dendrite arborization.
Hippocampal neurons were transfected at DIV1 with GFP alone or in combination with HA-IL1RAPL1, HA-IL1RAPL1 C31R, HA-PTPδ, HA-PTPδ ECTO and shRNA against PTPδ and were fixed and stained at DIV4 with anti HA and anti MAP2 antibodies (Fig. 5a). Quantification of total number of primary (Fig. 5b) and secondary dendrites, (Fig. 5c) and the total length of primary and

secondary dendrites (Fig. 5d) of neurons overexpressing IL1RAPL1 and PTPδ constructs was performed.

Also in immature neurons, the overexpression of IL1RAPL1 leads to a semplification of dendritic arborization: the total number of primary dendrites is lower respect the control (GFP overexpressing neurons) (Fig. 5b). For this effect, the extracellular domain of IL1RAPL1 is required. On the opposite, PTPδ ECTO overexpressing neurons present a more complex arborisation (Fig. 5b) and a higher length of primary and secondary dendrites (Fig. 5d). The expression of shRNA against PTPδ instead reduces the number of secondary dendrites and the total length of primary and secondary dendrites.

From these data we can conclude that the *trans*-synaptic interaction between IL1RAPL1 and PTPδ seems not to be involved in the IL1RAPL1-mediated effect on dendrite arborisation semplification, even if PTPδ seems to be involved in early neurite development (Wang and Bixby, 1999; Gonzalez-Brito and Bixby, 2006).

We then tried to understand wether a possible *cis*-interaction between IL1RAPL1 and PTPδ mediate the IL1RAPL1 effect on dendrite arborisation. We transfected neurons with GFP alone or with HA-IL1RAPL1, HA-PTPδ, HA-PTPδ ECTO, HA-PTPδ a-b- and shRNA against PTPδ and IL1RAPL1 in combination with HA-PTPδ, HA-PTPδ ECTO and HA-PTPδ a-b- at DIV 11, and we then fixed and stained neurons at DIV 14-15 with anti-HA and anti-IL1RAPL1 antibodies. Images obtained by confocal microscopy (Fig.6a) were subjected to Sholl analysis and the total number of branching points was quantified (Fig. 6b).

The analysis showed that the co-overexpression of PTPδ full length, PTPδ a-b- and PTPδ ECTO block the ability of overexpressed IL1RAPL1 to reduce the total number of branching points.

Otherwise the shRNA mediated silencing of PTPδ protein leads to the simplification of dendrite arborisation like the overexpression of IL1RAPL1 does.

We can conclude that a *cis*-interaction between IL1RAPL1 and PTPδ can occur in the dendrite compartment and can block the effect of IL1RAPL1 on dendrites. This antagonistic effect of PTPδ is confirmed also by the effect of its silencing that rescue the effect of IL1RAPL1 on dendrites. The extracellular part of PTPδ seems to be sufficient to block the IL1RAPL1 effect and the absence of PTPδ miniexons a and b doesn't affect the PTPδ capacity of IL1RAPL1 blockade. Possibly, the role of the miniexons in the IL1RAPL1-PTPδ interaction (Yoshida et al.) is less important in *cis* than in *trans*.

PTPδ and IL1RAPL1 *cis*-interaction

To better characterize the *cis*-interaction between IL1RAPL1 and PTPδ we decided to look at localization of overexpressed PTPδ on dendrite surface membrane in combination or not with overexpressed IL1RAPL1. Hippocampal neurons were transfected at DIV11 with GFP, HA-IL1RAPL1 and IL1RAPL1 in combination with HA-PTPδ and HA-PTPδa-b- (Fig. 7a). At DIV 14-15, live hippocampal neurons were stained for 10 min at 37°C with anti-HA-tag rabbit antibody to label overexpressed protein on the membrane surface. After washing, neurons were fixed and incubated with anti-HA-tag mouse antibody in permeabilizing condition. Then primary antibodies were labeled with FITC- and Cy3-conjugated secondary antibodies. The ratio of integrated intensity of surface HA signal per total HA signal was measured for each neuron, and the mean + SEM is shown in the graph (Fig. 7b).

Like already published (Pavlowsky et al., ; Ramos-Brossier et al.), IL1RAPL1 is clearly present in dendrites and on the cell surface, otherwise overexpressed PTPδ at DIV14 is mostly localized in the axon (Takahashi and Craig, ; Dunah et al., 2005) and the relative aboundance of protein on the dendrite cell surface is lower. The co-overexpression of IL1RAPL1 with PTPδ leads to an increased amount of PTPδ on the dendrite cell surface, showing a probable *cis*-interaction in dendrites between the two proteins.

PTPδ a-b- overexpressing neurons show a more equal distribution between axonal and dendrite compartments respect to PTPδ, but the distribution of the protein seems not to change with the co-overexpression of IL1RAPL1. This experiment suggests that PTPδ is recruited to dendrites by a *cis*-interaction with IL1RAPL1. The dendritic recruited PTPδ is probably able to block the effect of IL1RAPL1 on dendritic arborisation.

We can specuate that the *cis*-interaction is lower or absent between IL1RAPL1 and PTPδ a-b-.

Effect of IL1RAPL1 and PTPδ co-overexpression on synaptogenesis

The IL1RAPL1 mediated recruitment of presynaptic compartment was already evaluated using VGLUT1 and VGAT as markers of excitatory and inhibitory synapses (Pavlowsky et al., ; Ramos-Brossier et al., ; Valnegri et al.).

Knocking-down or overexpressing IL1RAPL1 decreases or increases excitatory synapse formation, respectively (Pavlowsky et al., ; Valnegri et al., ; Yoshida et al., ; Yoshida and Mishina, 2008). In order to evaluate the inhibitory effect of PTPδ mediated by the *cis*-interaction with IL1RAPL1 in dendrites, hippocampal neurons were transfected at DIV11 with GFP, HA-IL1RAPL1 and IL1RAPL1 alone or in combination with HA-PTPδ than fixed a DIV 14-15 and stained with anti-HA, anti-VGLUT1 and anti-VGAT antibodies (Fig. 8a and 9a). Quantification of VGLUT1 and

VGAT clusters intensity in neurons overexpressing IL1RAPL1 and PTPδ was evaluated (Fig. 8b and 9b).

Our results show, as expected that the overexpression of PTPδ full length doesn't affect the excitatory and inhibitory synapses recruitment. However, the co-overexpressed PTPδ blocks the IL1RAPL1 mediated recruitment of excitatory presynaptic compartment.

Thus our data indicate that the *cis*-interaction between IL1RAPL1 and PTPδ occurs at the extracellular domain and block the effect of IL1RAPL1 on dendrites and on pre-synaptic compartment.

IL1RAPL1-KO mice behavioral tests

3 months old IL1RAPL1-KO and WT mice undergo some behavioural test to asses if the knocking down of the protein can reflect some of the phenotypes typical of ID and ASD patients and thus if IL1RAPL1-KO mouse can be consider as a good model to study this pathologies.

Results from behavioural studies indicate that KO mice were healthy showing normal sensory abilites (olfactory test, Fig. 10), normal innate behaviour (nest building test, data not shown) and normal sociability features (data not shown).

In the olfactory test, the latency to find the buried palatable food is recorded and no differences were observed between WT and KO mice.

Mice were subjected to Passive Avoidance test. The time latency to learn the association between the shock and the dark compartment was measured (Fig. 11) and no difference was found between WT and IL1RAPL1-KO mice. This finding suggests that KO mice show a good performance in a test that measure cortical and hippocampal area activity.

The spontaneous motor activity of WT and KO mice was also evaluated (Fig. 12). KO animals are characterized by an increased number of horizontal and vertical beam breaks, thus presenting a clear hyperactivity feature respect WT mice.

The Morris water maze test was chosen to asses the mice capacity to remember the position of an hidden platform submerged below the water surface (Fig. 13). IL1RAPL1-KO mice need more time to find the platform respect WT mice, showing a decreased spatial memory capacity that suggests an altered hippocampal activity.

The Elevated Plus Maze paradigm was used to study anxiety related behavior (Fig. 14). The number of open- and closed-arm entries and the time spent in open and closed arms were recorded for 5 min. KO mice show a slight less anxiety compared to WT as shown by the increased time to remain in the open arm.

Western Blot analysis on WT, HET and IL1RAPL1 KO mice

To identify possibly major synaptic biochemical alteration in the IL1RAPL1 KO mice total brain, lysate and crude synaptosomal fraction from 3 months old female WT, HET and IL1RAPL1 KO mice were analysed by Western Blot using a panel of antibodies against pre- and post-synaptic proteins (Fig. 15). We didn't find any major difference in synaptic protein expression between the genotypes.

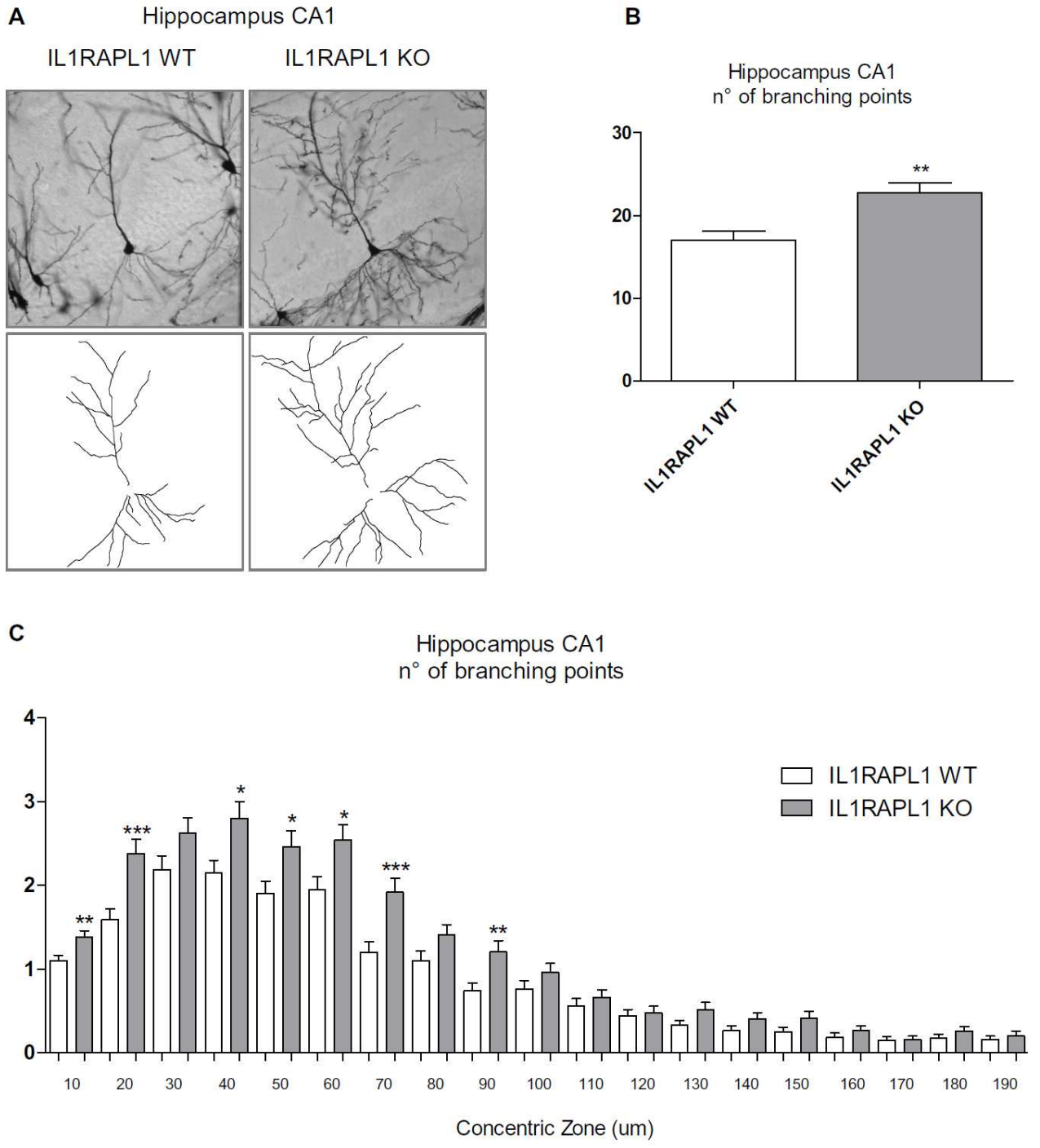


Fig. 1. Dendritic arborization complexity of CA1 hippocampal neurons is altered in IL1RAPL1-KO adult mice

(A) Representative images of Golgi stained brain from WT (on the left) and IL1RAPL1 KO mice (on the right) and a schematization of their dendrites below.

(B) Quantification of total number of neuronal branching points from WT and IL1RAPL1-KO mice (8 neurons analyzed for each mice, N=9 for each genotype). Data are shown as mean of number of branching points ± SEM, *p<0.05, **p < 0.005, student's t-test.

(C) Quantification of the number of branching points from WT and IL1RAPL1-KO mice plotted on the distance from the soma (µm). All values represent mean of total branching points every 10 µm ± SEM, *p<0.05, **p<0.005, ***p<0.001, student's t-test.

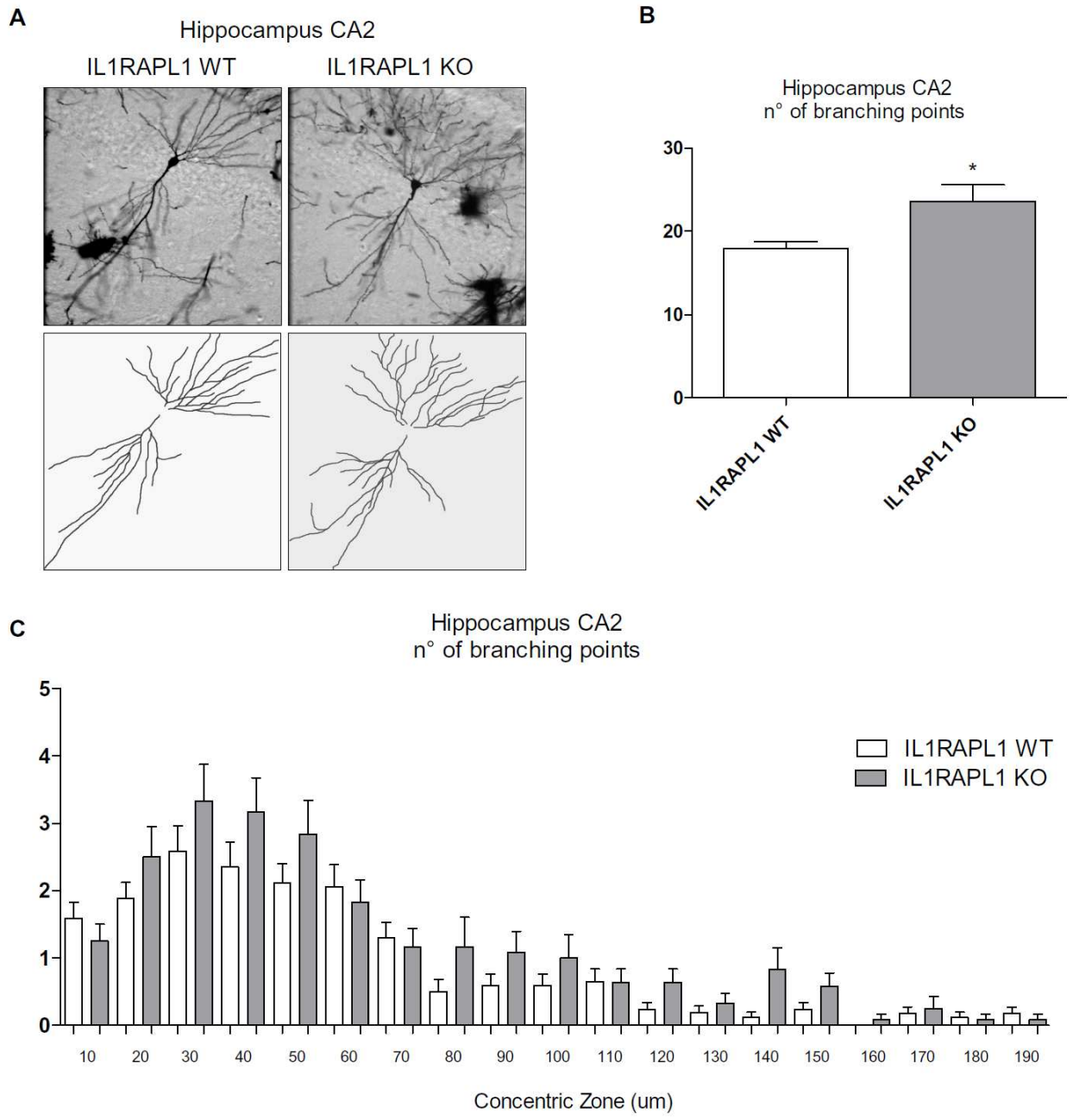


Fig. 2. Dendritic arborization complexity of CA2 hippocampal neurons is altered in IL1RAPL1-KO adult mice.

(A) Representative images of Golgi stained brain from WT (on the left) and IL1RAPL1 KO mice (on the right) and a schematization of their dendrites below.

(B) Quantification of total number of neuronal branching points from WT and IL1RAPL1-KO mice (8 neurons analyzed for each mice, N=4 for each genotype). Data are shown as mean of number of branching points ± SEM, *p<0.05, student's t-test.

(C) Quantification of the number of branching points from WT and IL1RAPL1-KO mice plotted on the distance from the soma (µm). All values represent mean of total branching points every 10 µm ± SEM.

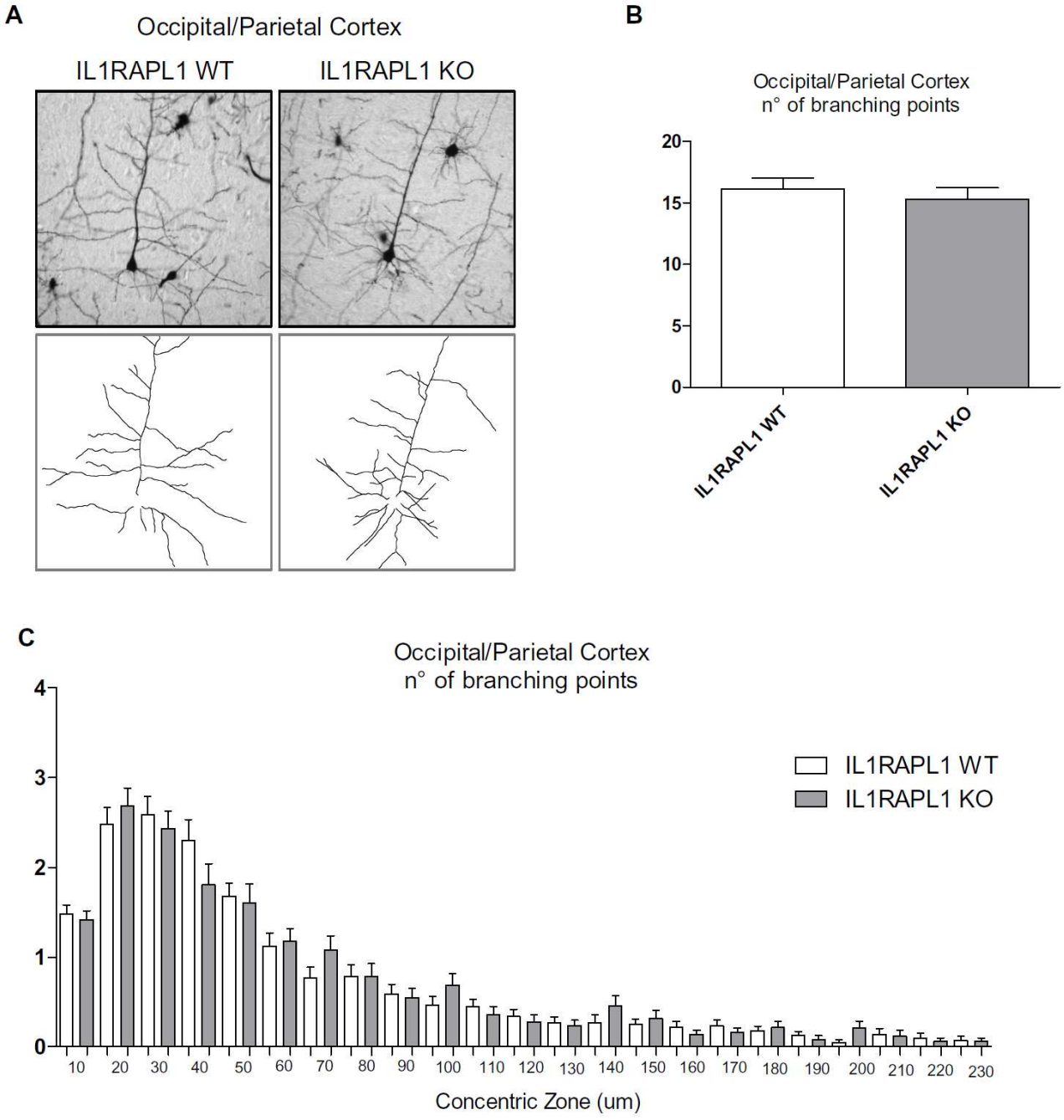


Fig. 3. WT and IL1RAPL1-KO adult mice dendritic arborization complexity in Occipital and Parietal cortex.

(A) Representative images of Golgi stained brain from WT (on the left) and IL1RAPL1 KO mice (on the right) and a schematization of their dendrites below.

(B) Quantification of total number of neuronal branching points from WT and IL1RAPL1-KO mice (8 neurons analyzed for each mice, N=7 for each genotype). Data are shown as mean of number of branching points ± SEM.

(C) Quantification of the number of branching points from WT and IL1RAPL1-KO mice plotted on the distance from the soma (µm). All values represent mean of total branching points every 10 µm ± SEM.

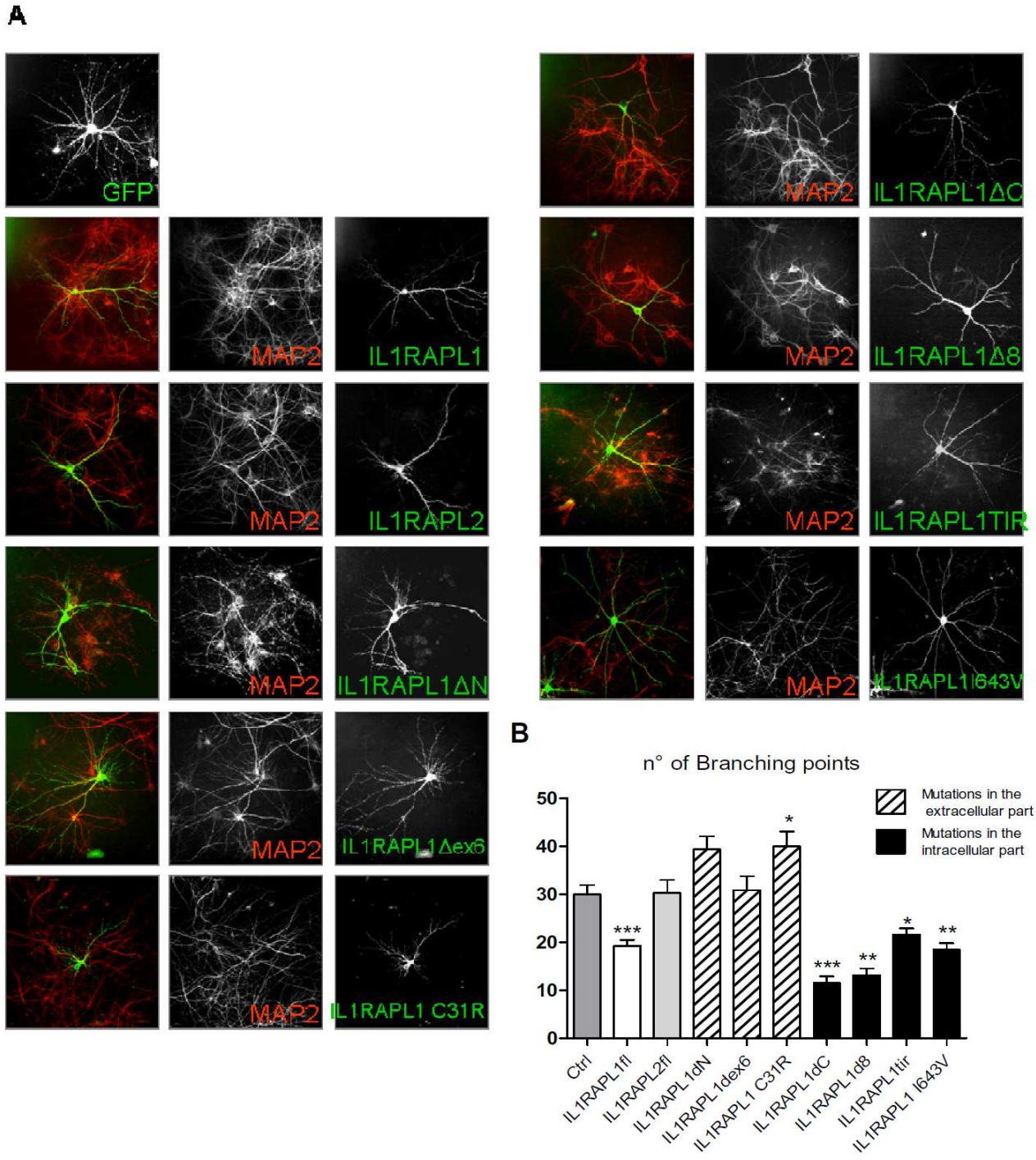
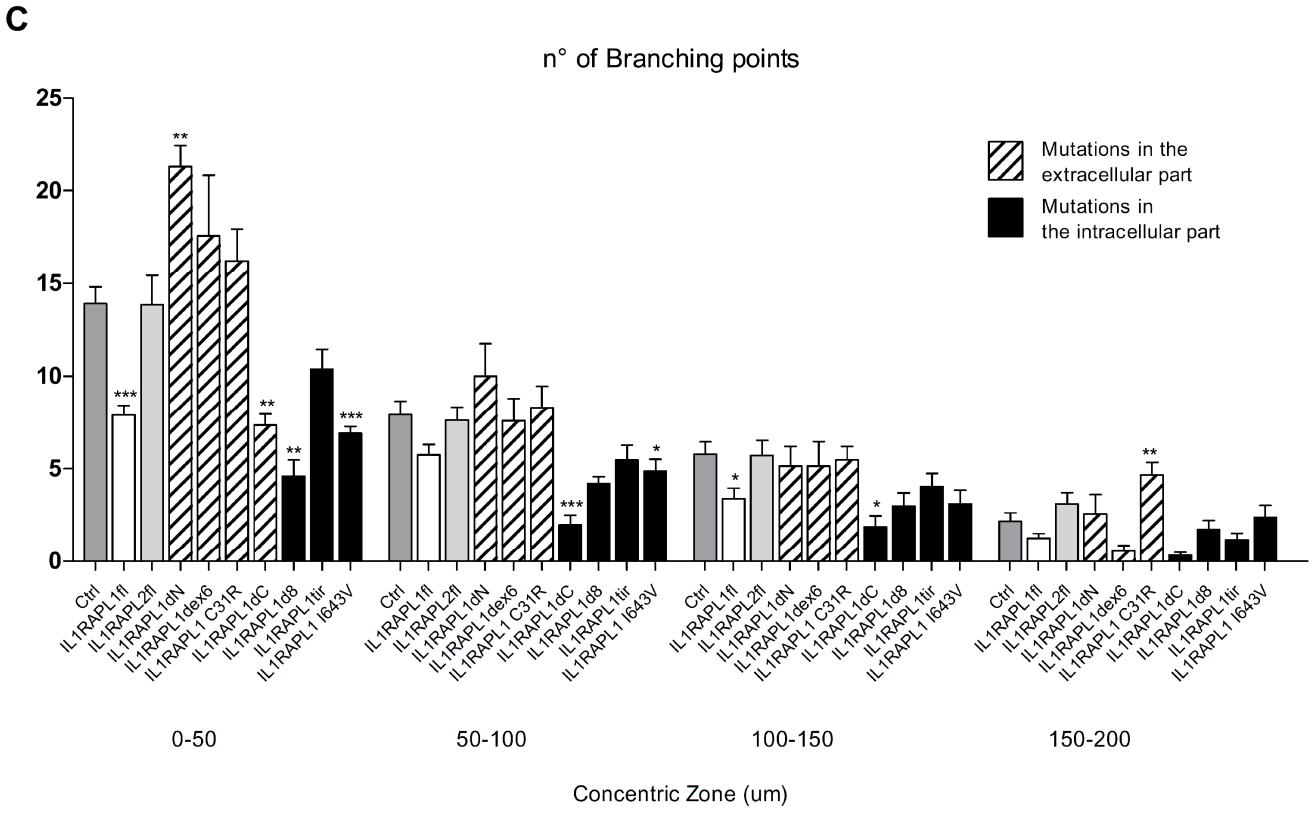


Fig. 4. Dendritic arborization complexity quantification in neuronal primary colture.

(A) Hippocampal neurons were transfected at DIV11 with GFP, HA-IL1RAPL1, HA-IL1RAPL2, HA-IL1RAPL1ΔN, HA-IL1RAPL1Δexon6, HA-IL1RAPL1 C31R, HA-IL1RAPL1ΔC, HA-IL1RAPL1Δ8, HA-IL1RAPL1TIR or HA-IL1RAPL1 I643V and were fixed and stained at DIV14 with anti HA and anti MAP2 antibodies. Each row of images (except for GFP one) shows double labeling for MAP2 (red, middle panel) and HA (green, right panel); merge is shown in color, in left panel.

(B) Quantification of total number of branching points of neurons overexpressing IL1RAPL1 constructs (15 neurons analyzed for each construct in three independent experiments). All values represent mean ± SEM, *p<0.05, **p<0.005, ***p < 0.001, ANOVA followed by Dunnet's post-hoc test.

(C) Quantification of number of branching points of neurons overexpressing IL1RAPL1 constructs plotted on the distance from the soma (µm). All values represent mean of total branching points every 50 µm ± SEM, *p<0.05, **p<0.005, ***p < 0.001, ANOVA followed by Dunnet's post-hoc test.



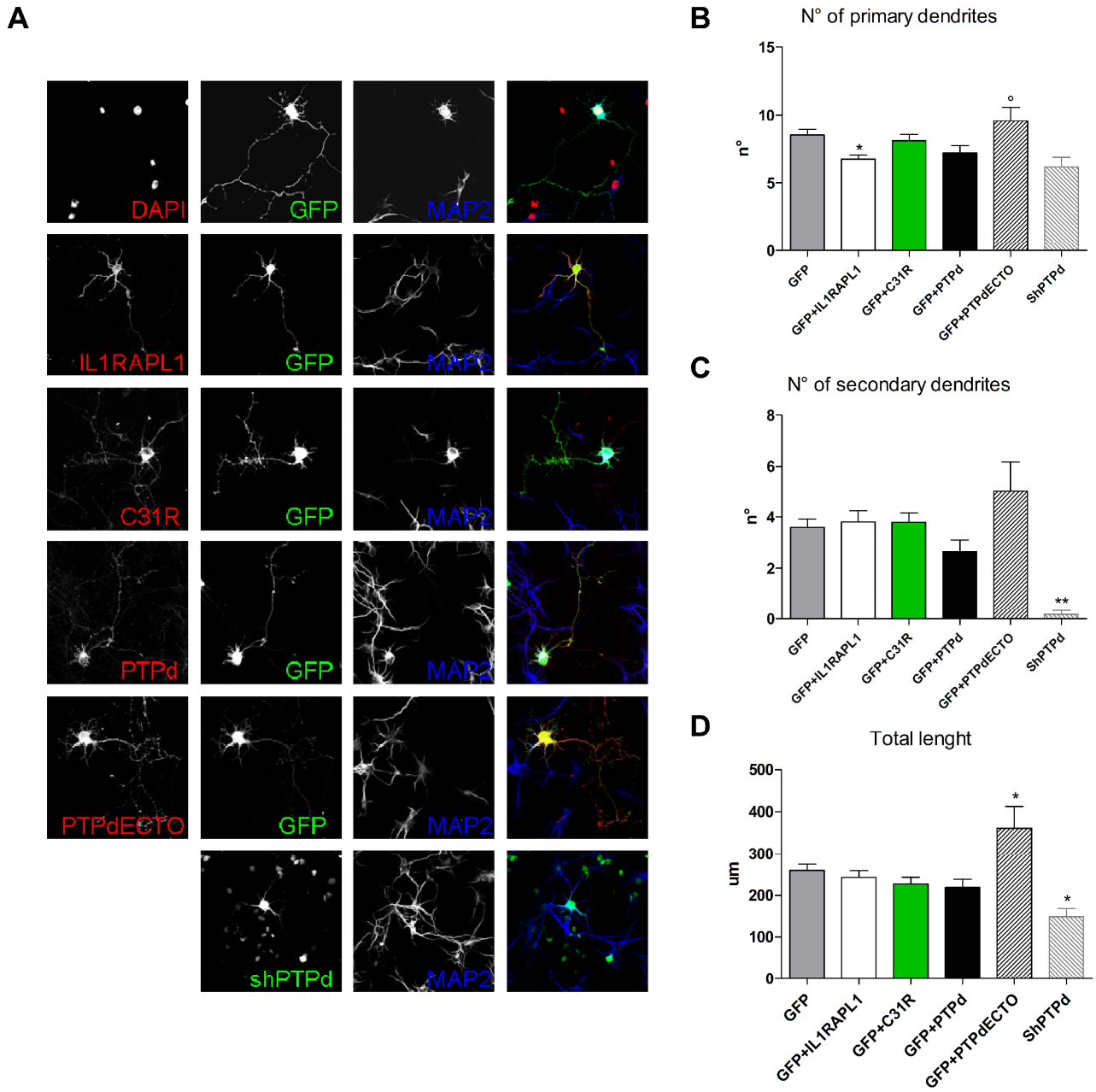


Fig. 5. Dendritic arborization complexity quantification in immature neurons.

(A) Hippocampal neurons were transfected at DIV1 with GFP, HA-IL1RAPL1, HA-IL1RAPL1 C31R, HA-PTPδ, HA-PTPδ ECTO and shRNA against PTPδ and were fixed and stained at DIV4 with anti HA and anti MAP2 antibodies. Each row of images (except for GFP one) shows GFP signal and labeling for MAP2 (blue) and HA (red); merge is shown in color, in right panel.

Quantification of total number of primary dendrites, (B) secondary dendrites, (C) total length of primary and secondary dendrites of neurons (D) overexpressing IL1RAPL1 and PTPδ constructs (15 neurons analyzed for each construct in three independent experiments). All values represent mean ± SEM, *p<0.05, **p<0.005, compared to control °p<0,005 respect to IL1RAPL1 full lenght (ANOVA followed by Tukey's post-hoc test).

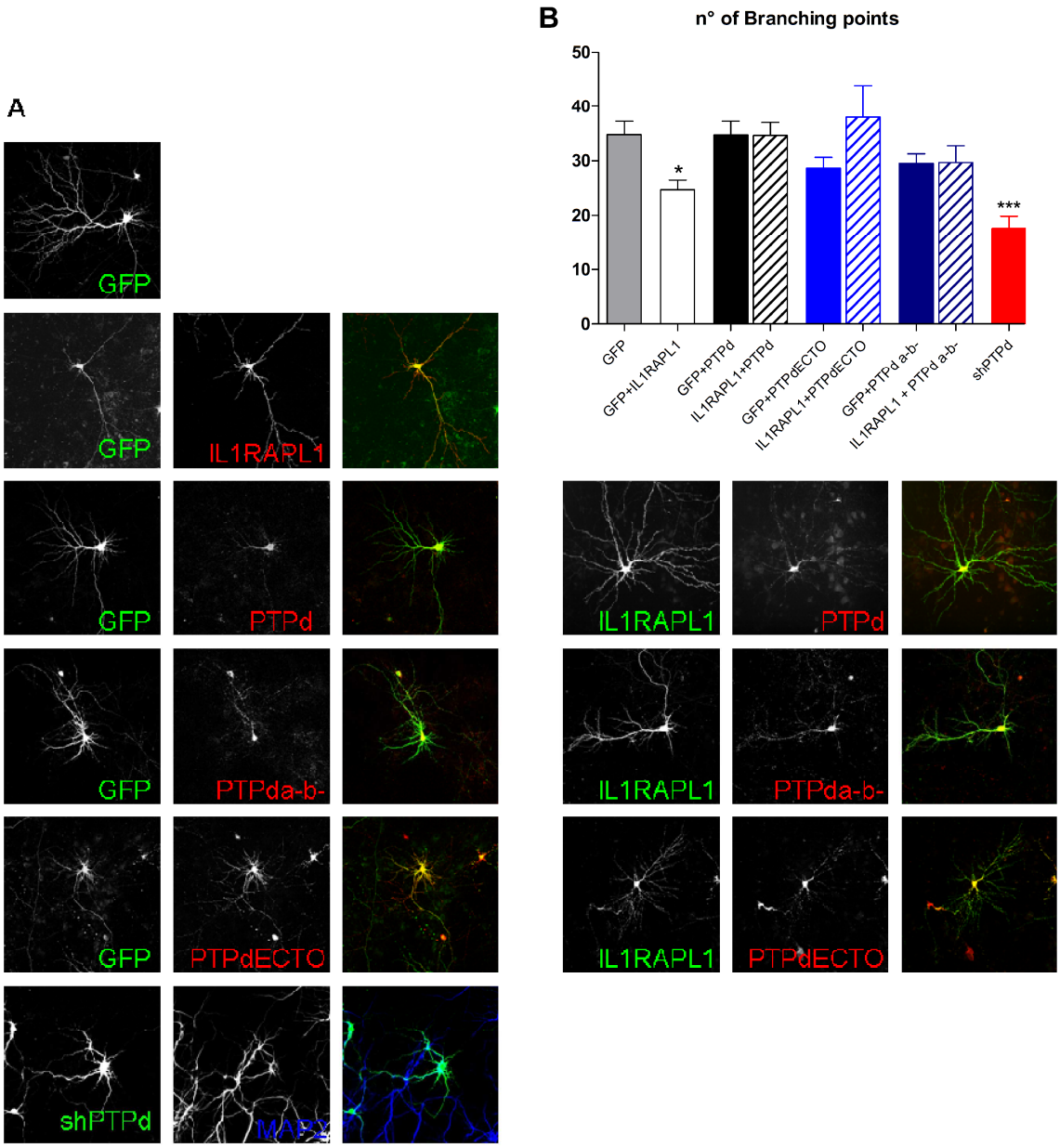


Fig. 6. Dendritic arborization complexity quantification in neuronal primary colture.

(A) Hippocampal neurons were transfected at DIV11 with GFP, IL1RAPL1, PTPδ constructs and shRNA against PTPδ and were fixed and stained at DIV15 with anti HA (red) and anti-IL1RAPL1 (green).

(B) Quantification of total number of branching points of neurons overexpressing IL1RAPL1 and PTPδ constructs (15 neurons analyzed for each construct in three independent experiments). All values represent mean ± SEM, *p<0.05, ** *p* <0.005, ***p < 0.001 (ANOVA followed by Tukey's post-hoc test).

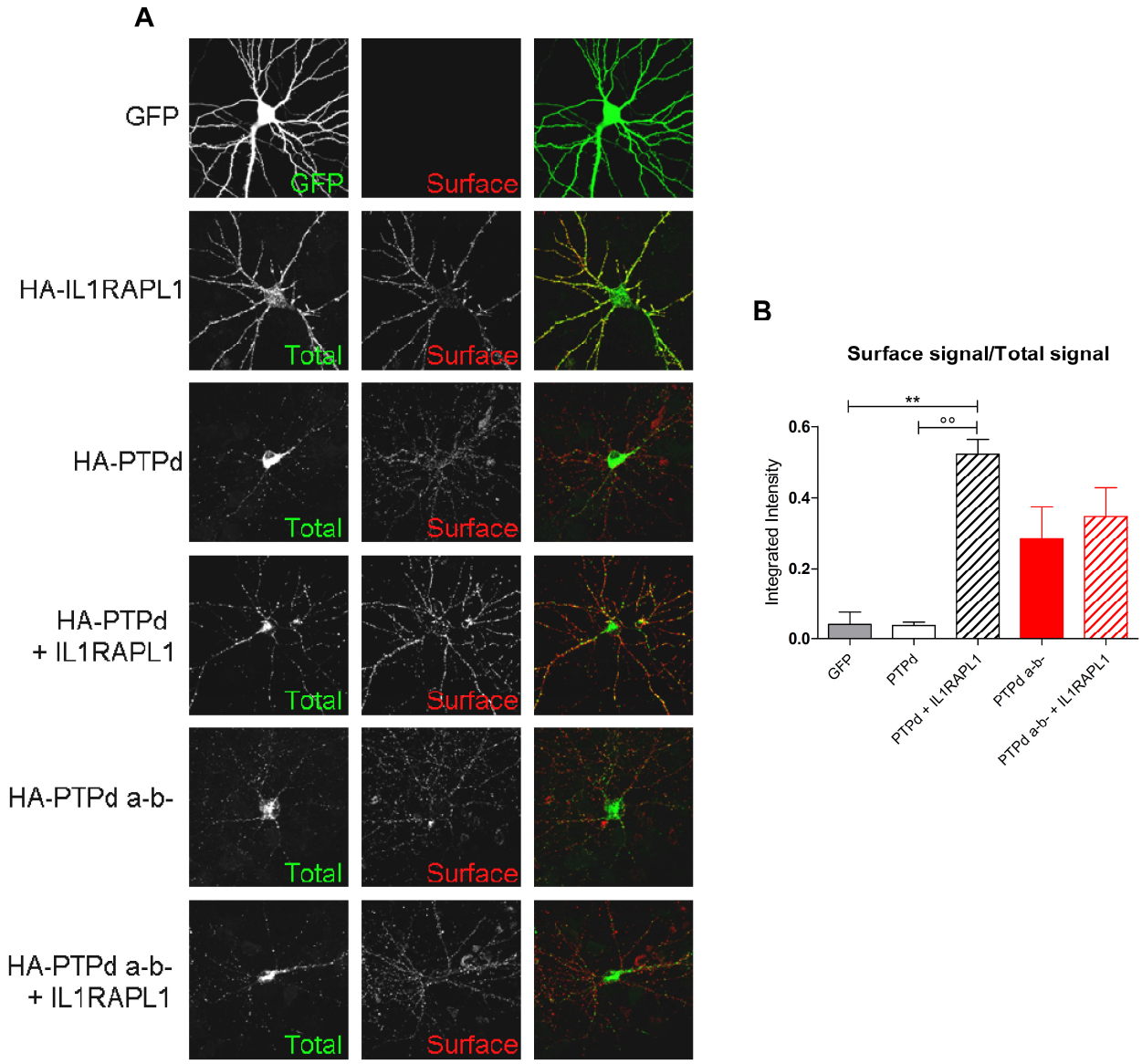


Fig. 7. PTPδ and IL1RAPL1 *cis*-interaction.

(A) Total (left panel) and surface (middle panel) staining of HA-PTPδ and HA-PTPδa-b- in mature hippocampal neurons using an anti-HA tag antibody. The ratio of integrated intensity of surface HA signal per total HA signal was measured for each neuron, and the mean + SEM is shown in (B). All values represent mean ± SEM, **p<0.005, compared to control °°p<0,005 respect to PTPδ (ANOVA followed by Tukey's post-hoc test).

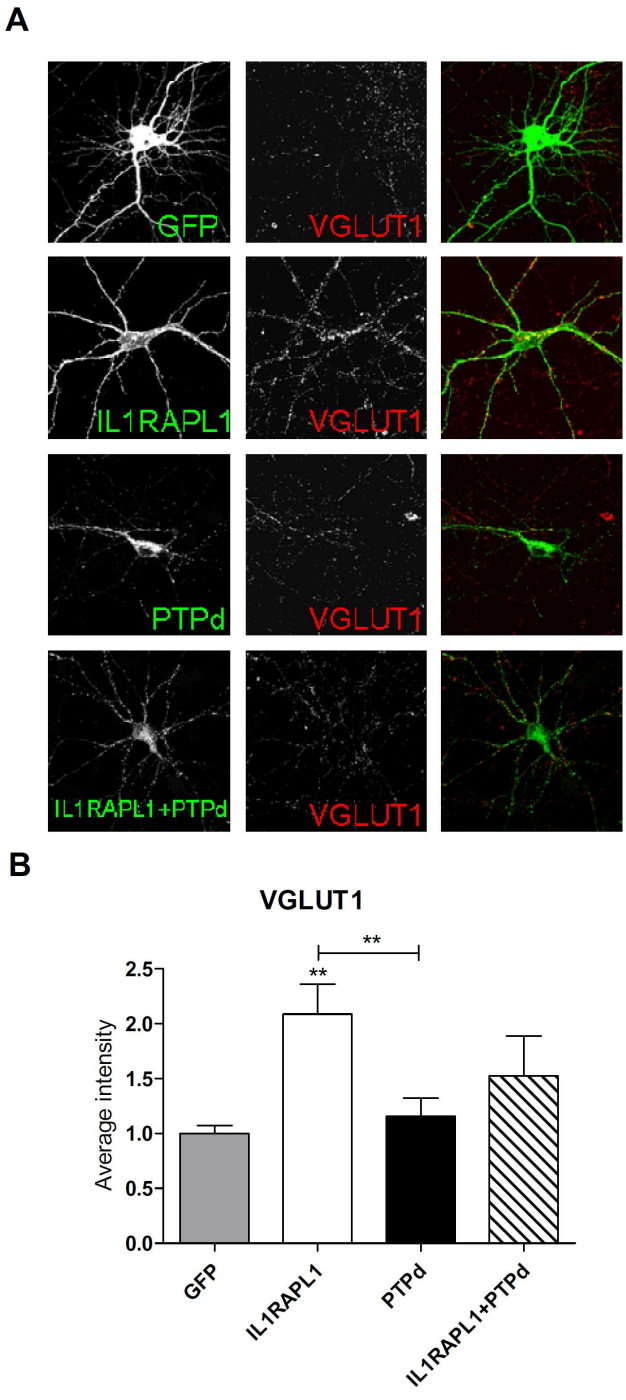


Fig. 8. Effect of IL1RAPL1 and PTPδ co-overexpression on excitatory synapse formation.

(A) Rat hippocampal neurons transfected with GFP, IL1RAPL1, HA-PTPδ and stained with anti-VGLUT1 antibody to label excitatory pre-synapses. Images show labeling for GFP (green), IL1RAPL1 (green), HA (green) and VGLUT1 (red); the merged images are shown in the right panel.

(B) Quantification of VGLUT1 clusters intensity. Bar graphs show the mean + SEM of VGLUT1 intensity (15 neurons from 3 independent experiments for each condition, ** p <0.005, compared to control neurons).

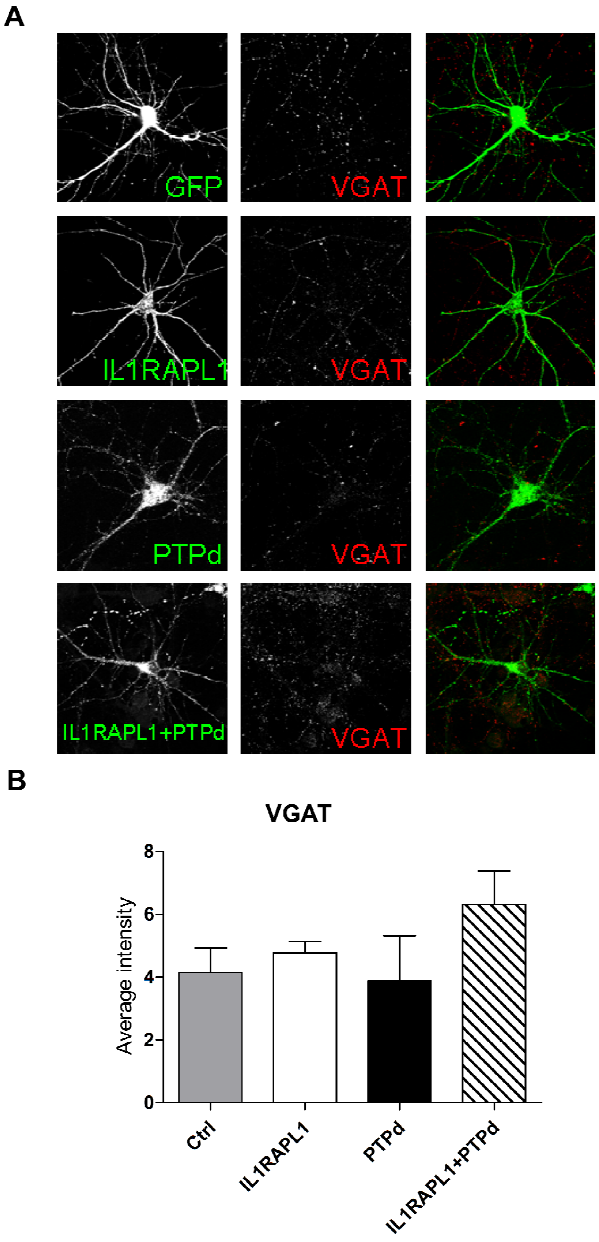


Fig. 9. Effect of IL1RAPL1 and PTPδ co-overexpression on inhibitory synapse formation.

(A) Rat hippocampal neurons transfected with GFP, IL1RAPL1, HA-PTPδ and stained with anti-VGAT antibody to label excitatory pre-synapses. Images show labeling for GFP (green), IL1RAPL1 (green), HA (green) and VGAT (red); the merged images are shown in the right panel.

(B) Quantification of VGAT clusters intensity. Bar graphs show the mean + SEM of VGAT intensity (15 neurons from 3 independent experiments for each condition).

N= 3-5

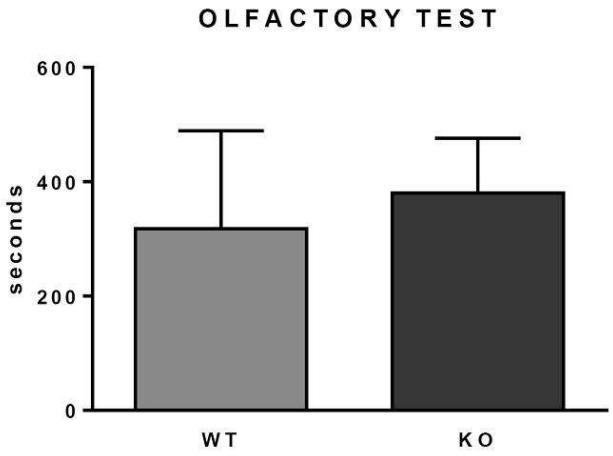


Fig. 10. Mice behavior: Olfactory test (Data obtained in collaboration with Luisa Ponzoni from Maria Elvina Sala Laboratory). Latency to find an hidden palatable food is recorded and plotted in seconds ± SEM.

N= 10

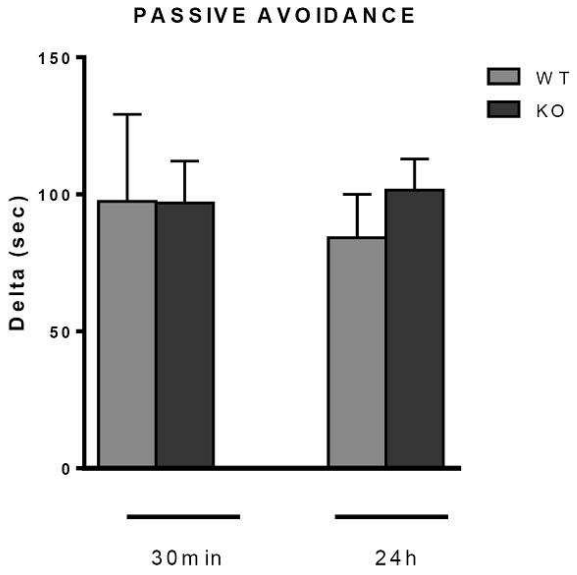


Fig. 11. Mice behavior: Passive avoidance test (Data obtained in collaboration with Luisa Ponzoni from Maria Elvina Sala Laboratory). The test apparatus consisted of one light and one dark compartments, connected via a sliding door. In the acquisition trial, each mouse was placed in the light compartment and allowed to enter the dark compartment; the time (in s) taken to do so was recorded. Once the mouse was in the dark compartment, the sliding door was closed and an unavoid- able electric shock delivered via the paws. The animal was then placed back in the home cage until the retention trial. The retention trial was carried out 30 min or 24 h after the acquisition trial, by positioning the mouse in the light compartment and recording the time taken to enter the dark compartment. Δsec are plotted ± SEM.

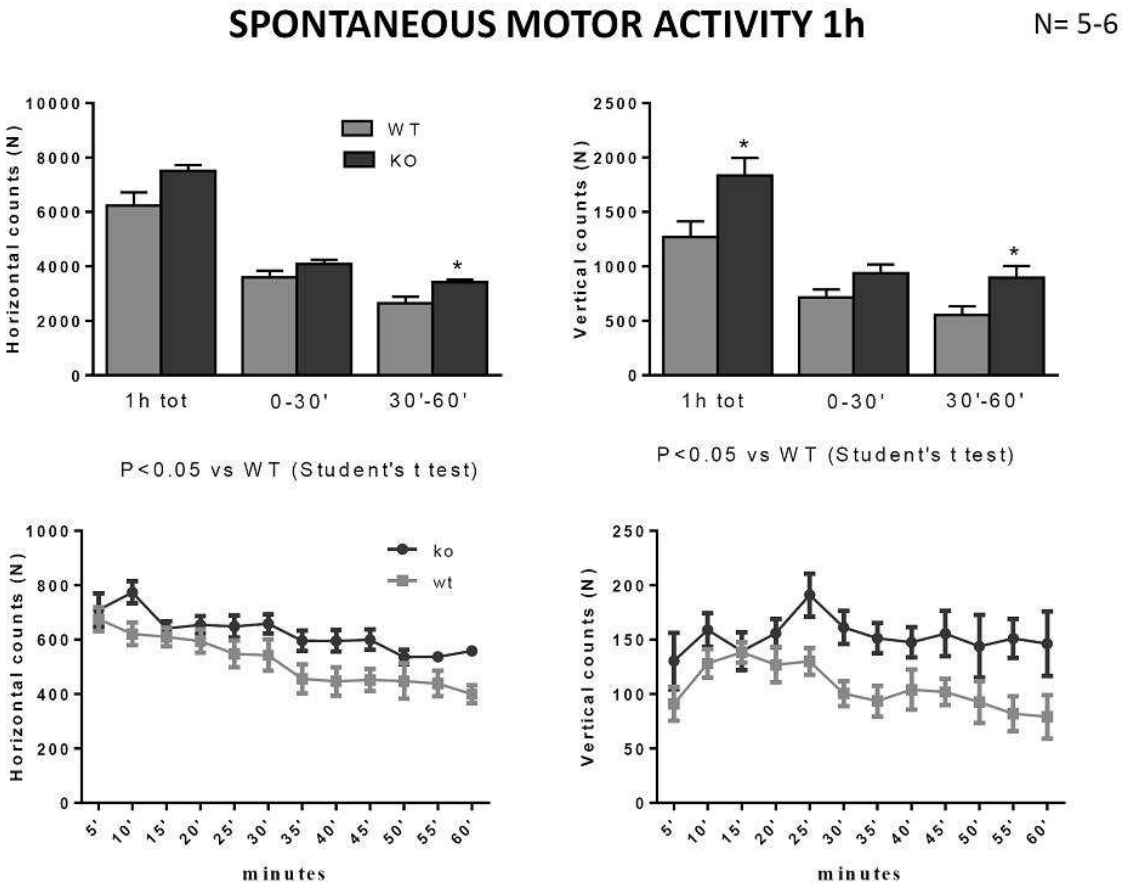


Fig. 12. Mice behavior: spontaneous motor activity (Data obtained in collaboration with Luisa Ponzoni from Maria Elvina Sala Laboratory). The spontaneous motor activity of WT and KO mice was evaluated: cumulative horizontal and vertical beam breaks were counted for 60 min. In the upper raw of graph the total number of horizontal and vertical mouvements are plotted together or divided in 30 minutes time intervals. In the bottom raw of graph, the counts are plotted on the minuts.

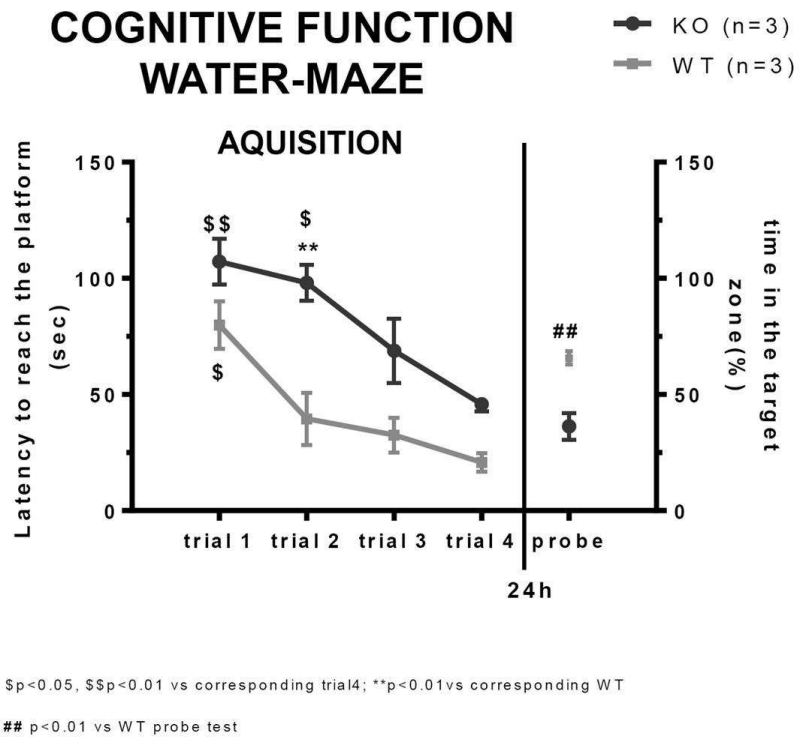


Fig. 13. Mice behavior: Morris water maze (Data obtained in collaboration with Luisa Ponzoni from Maria Elvina Sala Laboratory). The escape latency and time spent in the target zone of the maze were manually recorded.

Fig. 14. Mice behavior: Elevated Plus Maze (Data obtained in collaboration with Luisa Ponzoni from Maria Elvina Sala Laboratory). The Elevated Plus Maze paradigm was used to study anxiety related behavior. The number of open- and closed-arm entries and the time spent in open and closed arms were recorded for 5 min.

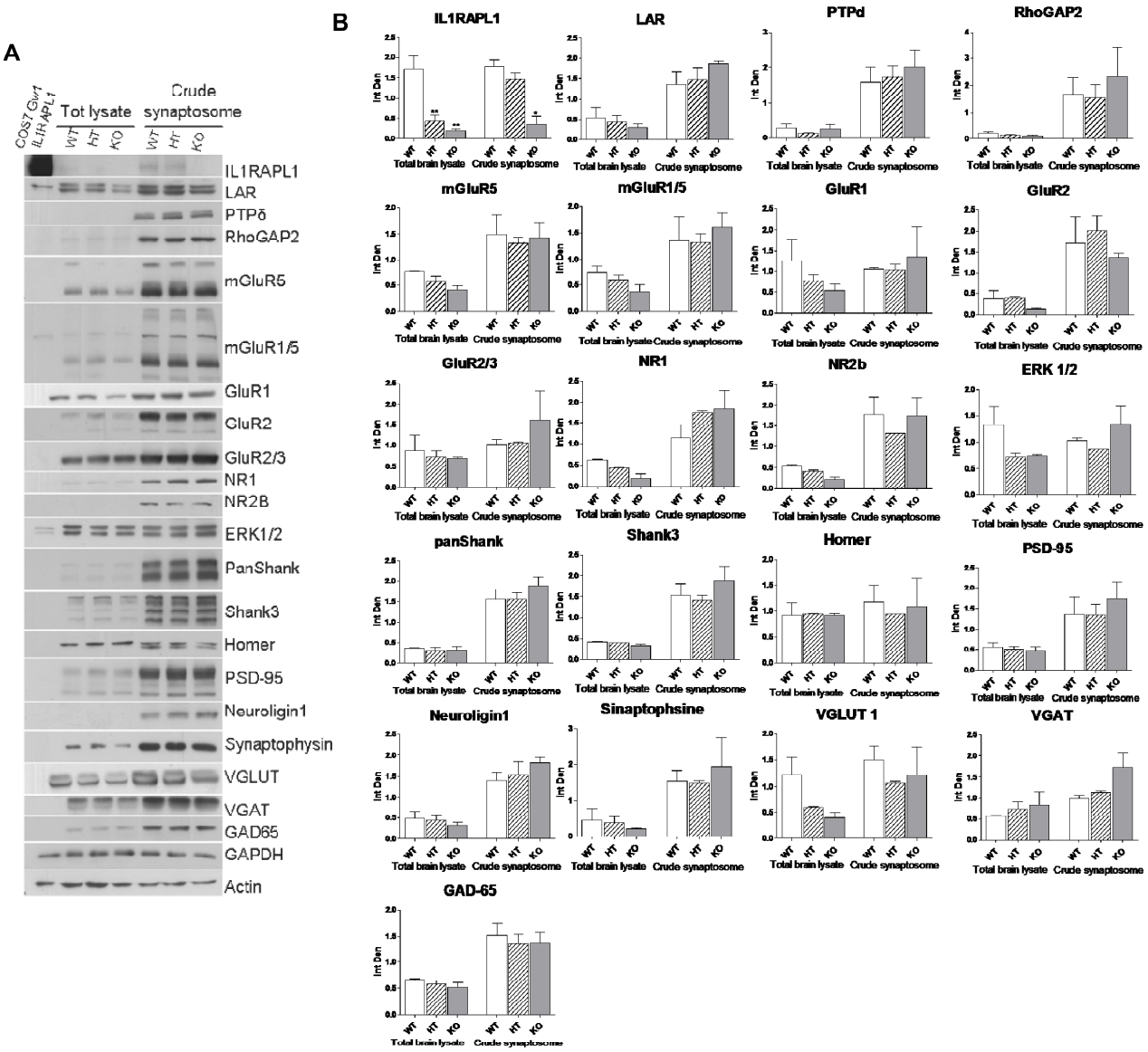


Fig. 15. Western Blot analysis on total brain lysate and crude synaptosomal fraction from WT, HET and IL1RAPL1 KO mice.

(A) Western Blot analysis of 3 months old female WT, HET and IL1RAPL1 KO mice.

(B) Quantification of the integrated density of the bands, normalized on Actin ± SEM (ANOVA followed by Tukey's post-hoc test, * p <0.05, ** p <0.005, ***p < 0.001 compared to WT mice).

DISCUSSION

NOVEL *IL1RAPL1* MUTATIONS ASSOCIATED WITH INTELLECTUAL DISABILITY IMPAIR SYNAPTOGENESIS

Discussion of the results about the function of the three novel independent mutations of *IL1RAPL1* gene IL1RAPL1Δexon6, IL1RAPL1 C31R and IL1RAPL1 I643V has already been published (Ramos-Brossier et al.), thus it is not reported in this Thesis.

THE X-LINKED INTELLECTUAL DISABILITY PROTEIN IL1RAPL1 REGULATES DENDRITIC ARBORIZATION

Dendrites must satisfy many physiological requirements to ensure proper neuronal function. Numerous molecules and pathways are involved in the correct dendrite development, maintainment and function. For instance, actin and microtubules are the major structural components that underlie dendrite morphology and regulators of actin and microtubule dynamics therefore have important roles in dendrite morphogenesis (Leemhuis et al., 2004; Newey et al., 2005; Chen and Firestein, 2007; Sfakianos et al., 2007). Furthermore, *in vivo* studies indicate that synaptic activity promotes dendritic arbor elaboration and stabilization (Cline, 2001). In some cases, blocking synaptic activity induces an increase of dendritic growth. For example, blocking glutamate receptors or L-type calcium channels increases dendritic growth of pyramidal and nonpyramidal neurons in cultured slices of ferret visual cortex (McAllister, 2000). Blocking NMDA receptors in neonatal ferrets in vivo also increases dendritic branching and spine formation in neurons in the lateral geniculate nucleus (Rocha and Sur, 1995). Thus, the effects of neuronal activity and molecular mechanisms on dendritic morphology may be site- and context-dependent and may differ between animal species and between brain regions (McAllister, 2000).

Consistent with the necessity of appropriate dendritic architecture for high order brain functions, (including learning and memory), deficiencies in the architecture of dendrites have been observed in a variety of neurodevelopmental, neurodegenerative and neuropsychiatric disorders such as schizophrenia, Down's syndrome, fragile X syndrome, Angelman's syndrome, Rett's syndrome and autism (Verpelli et al., ; Miller and Kaplan, 2003; Bagni and Greenough, 2005; Pardo and Eberhart, 2007; Ramocki and Zoghbi, 2008; Walsh et al., 2008; Bourgeron, 2009).

Decreased dendritic branching in CA1 and CA4 hippocampal neurons occurs in patients with autism and neurons from patients with Rett syndrome. Similarly, altered dendritic arborization and decreased expression of glutamate receptors have been observed in CA3 hippocampal neurons of patients with schizophrenia (Kolomeets et al., 2007). Thus, an abnormality in the dendritic arbor is a common theme seen in disorders of the central nervous system and these aberrations may be directly linked to deficiencies in higher order brain functions (Arikkath).

Mutations in *IL1RAPL1* gene have been found in patients with ID or autism (Carrie, 1999; Piton, 2008). Thus we decided to study the neuronal morphology in IL1RAPL1 KO mice and rat neuron primary culture overexpressing IL1RAPL1 or IL1RAPL1 mutants to clarify how any changes in IL1RAPL1 function can lead to development of cognitive disorders in humans.

Piton et al. have characterized the functional impact of the I367SfsX6 mutation on IL1RAPL1 activity. The authors first carry on some expression studies of mouse *IL1RAPL1* gene and found the highest level of expression of IL1RAPL1 in the developing and post-natal hippocampus (Carrie et al., 1999), a structure involved in memory that have been shown to be altered in ASD patients (Cody et al., 2002). In cultures of rat hippocampal neurons IL1RAPL1 endogenous protein is present in the cell body but also in growth cones of short, dendrite-like processes. To determine the effect of the I367SfsX6 mutation on ILRAPL1 function in HEK293 cells, Piton et al. knocked down the expression of the endogenous ILRAPL1 using specific miRNAs. The knock-down of IL1RAPL1 leads to a significant increase in both the number and the length of neuritis. Cotransfection of the anti-endogenous IL1RAPL1 miRNA together with the full-length wild-type IL1RAPL1 cDNA restored the expression of IL1RAPL1 and rescued the neurite outgrowth activity to control levels. In contrast, cotransfection of the miRNA together with the IL1RAPL1 I367SfsX6 mutant cDNA resulted in cells with high neurite number and length, similar to cells transfected with the miRNA alone. Therefore, the mutated IL1RAPL1 protein was unable to rescue ILRAPL1 knockdown. This observation shows that the 7 bp deletion results in a loss of IL1RAPL1 function. Moreover, immunofluorescence studies using an antibody directed against IL1RAPL1 protein revealed a cytoplasmic pool of mutant protein but did not detect its presence at the cell surface, suggesting that the truncated form of IL1RAPL1 is mislocalized. Surprisingly, transfection of rat hippocampal neurons with either the human WT or the mutant IL1RAPL1 cDNA alone did not affect neurite outgrowth. In conclusion, Piton et al. demonstrated a role of IL1RAPL1 in neurite outgrowth.

Furthermore, Gambino et al. (Gambino et al., 2007) reported that the expression of IL1RAPL1 in PC12 cells, a neuron-like cell line in which it is not normally expressed, decreases neurite

outgrowth by inhibiting N-type voltage-gated calcium channels via its physical interaction with NCS-1/FREQ.

Given the emerging role of IL1RAPL1 in neurite outgrowth we decided to investigate this effect *in vivo* and *in vitro*.

We performed an analysis of dendritic arborisation complexity on Golgi stained neurons of CA1 (Fig. 1), CA2 (Fig. 2) and Occipital and Parietal cortex (Fig. 3) in WT and IL1RAPL1 KO mice. We observed an increased number of branching points in IL1RAPL1-KO mice hippocampal neurons of CA1 and CA2 fields. On the contrary, the number of branching points of cortical neurons from WT and KO mice is comparable. These results suggest that the absence of IL1RAPL1 *in vivo* affects the correct dendrite development in the hippocampus, but not in the cortex.
We can speculate that the effects of the protein on dendritic morphology may be site-dependent, thus IL1RAPL1 and its hypothetical interactors can have different role in different brain areas.
Thus, it will be also interesting to study if the expression of synaptic and neuronal proteins is modified by the KO of *IL1RAPL1* in different brain area. Up to now, we didn't find any major differences in synaptic protein expression between KO, HET and WT animals (Fig. 15).

To understand wich IL1RAPL1 protein domains are involved in the effect on dendrite branching, we performed some *in vitro* experiments.
The overexpression of IL1RAPL1 drives to a reduction in number of branching points compared to the control (GFP expressing neurons). The overexpression of constructs that present mutations and deletions in the intracellular domains of IL1RAPL1 leads to a decreased number of branching points like the full length does. On the contrary, the overexpression of mutants in the extracellular domains seem not to have an effect on arborization or to lead to a higher dendrite complexity (IL1RAPL1 C31R overexpression) compared to the control.
Our data indicate that the extracellular part of IL1RAPL1 protein seems to be involved in this effect on dendrite arborization.
Given that the only known interactor of IL1RAPL1 extracellular domain is PTPδ and that the entire extracellular domain of PTPδ can function as a ligand to promote cell adhesion and neurite outgrowth (Gonzalez-Brito and Bixby, 2006), we decided to understand wether their *trans*-interaction could be involved in the effect on dendrite arborization.

In vitro, synaptogenesis process starts later than DIV 4 (Baj et al.), thus we decided to use young neurons to exclude the role of *trans*-synaptic interaction between IL1RAPL1 and PTPδ in the IL1RAPL1 mediated effect on dendrite arborization.

In young and synaptic immature neurons like in old ones, the overexpression of IL1RAPL1 leads to a semplification of dendritc arborization: the total number of primary dendrites is lower respect the control (GFP overexpressing neurons) (Fig. 5b). Moreover, PTPδ ECTO overexpressing neurons present a more complex arborisation (Fig. 5b) and a higher length of primary and secondary dendrites (Fig. 5b and 5d). The expression of shRNA against PTPδ seems to affect the number of secondary dendrites and the total length of primary and secondary dendrites.

From these data we can conclude that the *trans*-synaptic interaction between IL1RAPL1 and PTPδ seems not to be involved in the IL1RAPL1-mediated effect on dendrite arborisation simplification, even if PTPδ is involved in neurite development.

Given that the *trans*-synaptic interaction seems not to be involved, we tried to understand wether a possible *cis*-interaction between IL1RAPL1 and PTPδ could explain the IL1RAPL1-mediated effect on dendrite arborisation. For this porpoise, we transfected neurons with IL1RAPL1 plus PTPδ constructs in mature neurons (transfection at DIV 11 and staining at DIV 14-15). Surprisingly, we found that the co-overexpression of PTPδ constructs block the effect of overexpressed IL1RAPL1 on dendrite development. On the other hand, the shRNA mediated silencing of PTPδ protein leads to the simplification of dendrite arborisation like the overexpression of IL1RAPL1 does.

These data suggest that a *cis*-interaction between IL1RAPL1 and PTPδ can occur and can block the effect of IL1RAPL1 on dendrites. This antagonistic effect of PTPδ also suggested by the effect of PTPδ silencing mimics the effect of IL1RAPL1 overexpression. Possibly, the absence of endogenous PTPδ releases the activity on dendrites of endogenous IL1RAPL1. The extracellular part of PTPδ seems to be sufficient to block the IL1RAPL1 effect on dendrites because PTPδ ECTO is enough to block IL1RAPL1 activity. However we cannot completely explain why PTPδ without miniexons a and b, that should not binds to IL1RAPL1, is also able to block I1RAPL1 activity. It is possible that the role of the miniexons in the IL1RAPL1-PTPδ interaction (Yoshida et al.) is less important in *cis* than in *trans*.

Two experiments further demonstrate the abilty of PTPδ to interfere with IL1RAPL1 activity on dendrites.

First we studied the distribution of overexpressed PTPδ in dendrites plus or minus IL1RAPL1. We found that, like already published (Pavlowsky et al., ; Ramos-Brossier et al.), IL1RAPL1 is clearly present in dendrites and on the cell surface, otherwise PTPδ overexpressing neurons show a mostly

axonal staining (Takahashi and Craig, ; Dunah et al., 2005) and the relative aboundance of protein on dendrite membrane surface is low. Instead the co-overexpression of PTPδ with IL1RAPL1 leads to an increased amount of PTPδ on dendrite membrane surface, showing the recruitment of PTPδ to dendrites mediate by a probable *cis*-interaction between the two proteins.
We also found that overexpressed PTPδ a-b-, unable to bind IL1RAPL1 (Yoshida et al.) is more diffuse and is also localized in dendrites staining respect to PTPδ, but the distribution of the protein seems not to change with the co-overexpression of IL1RAPL1.
Our data suggest that the *cis*-interaction is lower between IL1RAPL1 and PTPδ a-b-, however PTPδ a-b- is someway still able to block the effect of IL1RAPL1 on dendrites morphology.
We also analyzed the effect of IL1RAPL1 and PTPδ co-overexpression on synaptogenesis. The IL1RAPL1 mediated recruitment of presynaptic compartment was already evaluated using VGLUT1 and VGAT as markers of excitatory and inhibitory synapses (Pavlowsky et al., ; Ramos-Brossier et al., ; Valnegri et al.).
Knocking-down or overexpressing IL1RAPL1 decreases or increases excitatory synapse formation, respectively (Pavlowsky et al., ; Valnegri et al., ; Yoshida et al., ; Yoshida and Mishina, 2008).
Interestingly we found that the co-overexpression of IL1RAPL1 and PTPδ block the IL1RAPL1 mediated recruitment of excitatory presynaptic compartment.

Thus, our *in vitro* results suggest that IL1RAPL1 extracellalar domains play a role in dendrite morphology and that the *cis*-interaction with PTPδ is able to block the IL1RAPL1-induced semplification of dendrite branching.
Our hypothesis is that an unknown IL1RAPL1 interactor is involved in the effect on dendrite morphology, in particular, because only in hippocampus neurons of IL1RAPL1 KO mice the dendrite morphology is altered, we plan to search for an IL1RAPL1 interactor with a primary role in hippocampal functions development or manteinment.

Our results from behavioural studies on WT and IL1RAPL1 KO mice indicate that the absence of the protein doesn't affect the sensory abilites and the innate behaviour of the animals.
Interestingly, findings emerging from memory function tests suggest that KO mice are able of good performance in depending on cortical and hippocampal area tasks (Passive Avoidance test), but present an impairment in Spatial memory test (Morris water maze).
We can conclude that KO animals are charachterized by an essentially hippocampal altered activity.
Furthermore, IL1RAPL1-KO mice present a clear hyperactivity feature and a slight less anxiety compared to WT mice.

The learning deficiencies and memory declines observed in IL1RAPL1-KO mice (Houbaert et al., ; Pavlowsky et al., ; Yasumura et al., ; Gambino et al., 2009) mimic the symptoms of ID children with *IL1RAPL1* mutations. ID children with deletions in the *IL1RAPL1* gene have slow developmental milestones such as the onsets of walking and speech and require special education (Barone et al., ; Behnecke et al., ; Franek et al., ; Nawara et al., 2008).

The *IL1RAPL1* gene is also associated with ASDs (Youngs et al., ; Bhat et al., 2008; Piton et al., 2008). ASDs are characterized by impairments in appropriate reciprocal social interactions, impairments in verbal social communication and high levels of ritualistic repetitive behaviours (Crawley, 2004).

Interestingly, IL1RAPL1 knockout mice in the spontaneous motor activity test showed an enhanced locomotor activity, which may be interpreted that IL1RAPL1 knockout mice are more prone to be stereotypic in behavior and that hyperactive behaviour (Yasumura et al.) reported for ID patients with mutations in the *IL1RAPL1* gene (Barone et al., ; Behnecke et al., ; Franek et al., ; Nawara et al., 2008) was reproduced in IL1RAPL1 knockout mice.

However, the social interaction of IL1RAPL1 knockout mice was not different from WT animals (data not shown).

We also noted that anxiety was reduced in IL1RAPL1 knockout mice. To measure anxiety-like behaviour in mice, elevated plus maze test is widely used (Bishop and Lahvis, ; Crawley). The percentages of time on open arms and entry into open arms in IL1RAPL1 knockout mice were significantly higher than those of wild-type mice. Thus, anxiety-like behaviours of IL1RAPL1 knockout mice are consistently reduced.

Present study with mutant mice (Houbaert et al., ; Pavlowsky et al., ; Yasumura et al., ; Gambino et al., 2009) revealed that the ablation of IL1RAPL1 affects diverse brain functions including learning, memory, and anxiety. Thus, it is reasonable that multiple brain functions are affected by the Mutation (Yasumura et al.).

Human patients with *IL1RAPL1* mutations are classified as ID and/or ASDs (Barone et al., ; Behnecke et al., ; Franek et al., ; Youngs et al., ; Carrie et al., 1999; Tabolacci et al., 2006; Bhat et al., 2008; Nawara et al., 2008; Piton et al., 2008). It remains to be examined how neural circuits responsible for these mental disorders are mainly affected by *IL1RAPL1* mutations. Interestingly, model mice of Fragile X syndrome, the most common ID, show decreased anxiety, increased locomotor activity and mild or no deficits in spatial learning (Peier et al., 2000; Mineur et al., 2002; Eadie et al., 2009). Based on the fact that FMRP regulates activity-dependent local mRNA translation upon metabotropic glutamate receptor 5 (mGluR5) stimulation, trials for the

treatment of Fragile X syndrome using mGluR5 antagonists have begun (Yasumura et al.). An increasing number of trials focus on treatment of the underlying defect, via re-equilibration of the biochemical imbalance that results from genetic mutations (Picker and Walsh). IL1RAPL1 induces excitatory synapse formation by *trans*-synaptic interaction with PTPδ (Valnegri et al., ; Yoshida et al.) and controls AMPA receptor trafficking by interacting with Mcf2l (Hayashi et al.). The decrease of spine density will cause the imbalance of excitation and inhibition in multiple brain circuits.

Our study reported for the fist time that IL1RAPL1 also regulate dendrite morphogenesis specifically in the hippocampus. Thus we propose that same hippocampal deficits identified in mice and presumably also in human patients carring *IL1RAPL1* mutation are caused by altered dendrite arborization of hippocampal neurons.

Further investigation of the molecular mechanism of IL1RAPL1-mediated effect on synaptogenesis and dendrite morphology would identify potential drug targets and IL1RAPL1 knockout mice will be useful to assess the new possible treatments (Yasumura et al.).

REFERENCES

Allen NJ, Barres BA (2009) Neuroscience: Glia - more than just brain glue. Nature 457:675-677.
Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc Natl Acad Sci U S A 107:4389-4394.
Anderton BH, Callahan L, Coleman P, Davies P, Flood D, Jicha GA, Ohm T, Weaver C (1998) Dendritic changes in Alzheimer's disease and factors that may underlie these changes. Prog Neurobiol 55:595-609.
Arellano JI, Espinosa A, Fairen A, Yuste R, DeFelipe J (2007) Non-synaptic dendritic spines in neocortex. Neuroscience 145:464-469.
Arikkath J Molecular mechanisms of dendrite morphogenesis. Front Cell Neurosci 6:61.
Baas PW, Deitch JS, Black MM, Banker GA (1988) Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci U S A 85:8335-8339.
Bagni C, Greenough WT (2005) From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Nat Rev Neurosci 6:376-387.
Bahi N, Friocourt G, Carrie A, Graham ME, Weiss JL, Chafey P, Fauchereau F, Burgoyne RD, Chelly J (2003) IL1 receptor accessory protein like, a protein involved in X-linked mental retardation, interacts with Neuronal Calcium Sensor-1 and regulates exocytosis. Hum Mol Genet 12:1415-1425.
Baj G, Patrizio A, Montalbano A, Sciancalepore M, Tongiorgi E Developmental and maintenance defects in Rett syndrome neurons identified by a new mouse staging system in vitro. Front Cell Neurosci 8:18.
Barone C, Bianca S, Luciano D, Di Benedetto D, Vinci M, Fichera M Intragenic ILRAPL1 deletion in a male patient with intellectual disability, mild dysmorphic signs, deafness, and behavioral problems. Am J Med Genet A 161A:1381-1385.
Bayes A, van de Lagemaat LN, Collins MO, Croning MD, Whittle IR, Choudhary JS, Grant SG Characterization of the proteome, diseases and evolution of the human postsynaptic density. Nat Neurosci 14:19-21.
Behnecke A, Hinderhofer K, Bartsch O, Numann A, Ipach ML, Damatova N, Haaf T, Dufke A, Riess O, Moog U Intragenic deletions of IL1RAPL1: Report of two cases and review of the literature. Am J Med Genet A 155A:372-379.
Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, Endris V, Roberts W, Szatmari P, Pinto D, Bonin M, Riess A, Engels H, Sprengel R, Scherer SW, Rappold GA Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet 42:489-491.
Bhat SS, Ladd S, Grass F, Spence JE, Brasington CK, Simensen RJ, Schwartz CE, Dupont BR, Stevenson RE, Srivastava AK (2008) Disruption of the IL1RAPL1 gene associated with a pericentromeric inversion of the X chromosome in a patient with mental retardation and autism. Clin Genet 73:94-96.
Bishop SL, Lahvis GP The autism diagnosis in translation: shared affect in children and mouse models of ASD. Autism Res 4:317-335.
Blanchetot C, Tertoolen LG, Overvoorde J, den Hertog J (2002) Intra- and intermolecular interactions between intracellular domains of receptor protein-tyrosine phosphatases. J Biol Chem 277:47263-47269.
Blundell J, Blaiss CA, Etherton MR, Espinosa F, Tabuchi K, Walz C, Bolliger MF, Sudhof TC, Powell CM Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J Neurosci 30:2115-2129.
Boda B, Mas C, Muller D (2002) Activity-dependent regulation of genes implicated in X-linked non-specific mental retardation. Neuroscience 114:13-17.
Born TL, Smith DE, Garka KE, Renshaw BR, Bertles JS, Sims JE (2000) Identification and characterization of two members of a novel class of the interleukin-1 receptor (IL-1R) family. Delineation of a new class of IL-1R-related proteins based on signaling. J Biol Chem 275:29946-29954.
Bourgeron T (2009) A synaptic trek to autism. Curr Opin Neurobiol 19:231-234.
Braida D, Sala M (2000) Cannabinoid-induced working memory impairment is reversed by a second generation cholinesterase inhibitor in rats. Neuroreport 11:2025-2029.
Broadbelt K, Jones LB (2008) Evidence of altered calmodulin immunoreactivity in areas 9 and 32 of schizophrenic prefrontal cortex. J Psychiatr Res 42:612-621.
Caceres A, Banker G, Steward O, Binder L, Payne M (1984) MAP2 is localized to the dendrites of hippocampal neurons which develop in culture. Brain Res 315:314-318.
Caldwell HK Neurobiology of sociability. Adv Exp Med Biol 739:187-205.
Carrie A, Jun L, Bienvenu T, Vinet MC, McDonell N, Couvert P, Zemni R, Cardona A, Van Buggenhout G, Frints S, Hamel B, Moraine C, Ropers HH, Strom T, Howell GR, Whittaker A, Ross MT, Kahn A, Fryns JP, Beldjord

C, Marynen P, Chelly J (1999) A new member of the IL-1 receptor family highly expressed in hippocampus and involved in X-linked mental retardation. Nat Genet 23:25-31.
Carter KC (2002) Early conjectures that Down syndrome is caused by chromosomal nondisjunction. Bull Hist Med 76:528-563.
Casanova JR, Nishimura M, Owens JW, Swann JW Impact of seizures on developing dendrites: implications for intellectual developmental disabilities. Epilepsia 53 Suppl 1:116-124.
Chahrour M, Jung SY, Shaw C, Zhou X, Wong ST, Qin J, Zoghbi HY (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320:1224-1229.
Chechlacz M, Gleeson JG (2003) Is mental retardation a defect of synapse structure and function? Pediatr Neurol 29:11-17.
Chen H, Firestein BL (2007) RhoA regulates dendrite branching in hippocampal neurons by decreasing cypin protein levels. J Neurosci 27:8378-8386.
Cline HT (2001) Dendritic arbor development and synaptogenesis. Curr Opin Neurobiol 11:118-126.
Cody H, Pelphrey K, Piven J (2002) Structural and functional magnetic resonance imaging of autism. Int J Dev Neurosci 20:421-438.
Coles CH, Shen Y, Tenney AP, Siebold C, Sutton GC, Lu W, Gallagher JT, Jones EY, Flanagan JG, Aricescu AR Proteoglycan-specific molecular switch for RPTPsigma clustering and neuronal extension. Science 332:484-488.
Couch BA, DeMarco GJ, Gourley SL, Koleske AJ Increased dendrite branching in AbetaPP/PS1 mice and elongation of dendrite arbors by fasudil administration. J Alzheimers Dis 20:1003-1008.
Craig AM, Banker G (1994) Neuronal polarity. Annu Rev Neurosci 17:267-310.
Crawley JN Translational animal models of autism and neurodevelopmental disorders. Dialogues Clin Neurosci 14:293-305.
Crawley JN (2004) Designing mouse behavioral tasks relevant to autistic-like behaviors. Ment Retard Dev Disabil Res Rev 10:248-258.
Crawley JN, Chen T, Puri A, Washburn R, Sullivan TL, Hill JM, Young NB, Nadler JJ, Moy SS, Young LJ, Caldwell HK, Young WS (2007) Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. Neuropeptides 41:145-163.
Dennis PB, Fumagalli S, Thomas G (1999) Target of rapamycin (TOR): balancing the opposing forces of protein synthesis and degradation. Curr Opin Genet Dev 9:49-54.
Dierssen M, Benavides-Piccione R, Martinez-Cue C, Estivill X, Florez J, Elston GN, DeFelipe J (2003) Alterations of neocortical pyramidal cell phenotype in the Ts65Dn mouse model of Down syndrome: effects of environmental enrichment. Cereb Cortex 13:758-764.
Dijkhuizen PA, Ghosh A (2005) BDNF regulates primary dendrite formation in cortical neurons via the PI3-kinase and MAP kinase signaling pathways. J Neurobiol 62:278-288.
Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7-61.
Dinopoulos A, Stefanou MI, Attilakos A, Tsirouda M, Papaevangelou V A case of startle epilepsy associated with IL1RAPL1 gene deletion. Pediatr Neurol 51:271-274.
Dunah AW, Hueske E, Wyszynski M, Hoogenraad CC, Jaworski J, Pak DT, Simonetta A, Liu G, Sheng M (2005) LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses. Nat Neurosci 8:458-467.
Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsater H, Sponheim E, Goubran-Botros H, Delorme R, Chabane N, Mouren-Simeoni MC, de Mas P, Bieth E, Roge B, Heron D, Burglen L, Gillberg C, Leboyer M, Bourgeron T (2007) Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 39:25-27.
Eadie BD, Zhang WN, Boehme F, Gil-Mohapel J, Kainer L, Simpson JM, Christie BR (2009) Fmr1 knockout mice show reduced anxiety and alterations in neurogenesis that are specific to the ventral dentate gyrus. Neurobiol Dis 36:361-373.
Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ (2008) Reversal of learning deficits in a Tsc2+/- mouse model of tuberous sclerosis. Nat Med 14:843-848.
Elia J, Gai X, Xie HM, Perin JC, Geiger E, Glessner JT, D'Arcy M, deBerardinis R, Frackelton E, Kim C, Lantieri F, Muganga BM, Wang L, Takeda T, Rappaport EF, Grant SF, Berrettini W, Devoto M, Shaikh TH, Hakonarson H, White PS Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. Mol Psychiatry 15:637-646.
Feldman R Oxytocin and social affiliation in humans. Horm Behav 61:380-391.
Feldmeyer D, Kask K, Brusa R, Kornau HC, Kolhekar R, Rozov A, Burnashev N, Jensen V, Hvalby O, Sprengel R, Seeburg PH (1999) Neurological dysfunctions in mice expressing different levels of the Q/R site-unedited AMPAR subunit GluR-B. Nat Neurosci 2:57-64.
Feller MB (1999) Spontaneous correlated activity in developing neural circuits. Neuron 22:653-656.

Fernandez AM, Jimenez S, Mecha M, Davila D, Guaza C, Vitorica J, Torres-Aleman I Regulation of the phosphatase calcineurin by insulin-like growth factor I unveils a key role of astrocytes in Alzheimer's pathology. Mol Psychiatry 17:705-718.

Flood DG (1991) Region-specific stability of dendritic extent in normal human aging and regression in Alzheimer's disease. II. Subiculum. Brain Res 540:83-95.

Franek KJ, Butler J, Johnson J, Simensen R, Friez MJ, Bartel F, Moss T, DuPont B, Berry K, Bauman M, Skinner C, Stevenson RE, Schwartz CE Deletion of the immunoglobulin domain of IL1RAPL1 results in nonsyndromic X-linked intellectual disability associated with behavioral problems and mild dysmorphism. Am J Med Genet A 155A:1109-1114.

Frank T, Rutherford MA, Strenzke N, Neef A, Pangrsic T, Khimich D, Fejtova A, Gundelfinger ED, Liberman MC, Harke B, Bryan KE, Lee A, Egner A, Riedel D, Moser T Bassoon and the synaptic ribbon organize Ca(2)+ channels and vesicles to add release sites and promote refilling. Neuron 68:724-738.

Froyen G, Van Esch H, Bauters M, Hollanders K, Frints SG, Vermeesch JR, Devriendt K, Fryns JP, Marynen P (2007) Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: important role for increased gene dosage of XLMR genes. Hum Mutat 28:1034-1042.

Fuerst PG, Koizumi A, Masland RH, Burgess RW (2008) Neurite arborization and mosaic spacing in the mouse retina require DSCAM. Nature 451:470-474.

Gambino F, Kneib M, Pavlowsky A, Skala H, Heitz S, Vitale N, Poulain B, Khelfaoui M, Chelly J, Billuart P, Humeau Y (2009) IL1RAPL1 controls inhibitory networks during cerebellar development in mice. Eur J Neurosci 30:1476-1486.

Gambino F, Pavlowsky A, Begle A, Dupont JL, Bahi N, Courjaret R, Gardette R, Hadjkacem H, Skala H, Poulain B, Chelly J, Vitale N, Humeau Y (2007) IL1-receptor accessory protein-like 1 (IL1RAPL1), a protein involved in cognitive functions, regulates N-type Ca2+-channel and neurite elongation. Proc Natl Acad Sci U S A 104:9063-9068.

Gao X, Xi G, Niu Y, Zhang S, Fu R, Zheng Z, Zhang K, Lv S, He H, Xue M, Zhang F (2008) A study on the correlation between IL1RAPL1 and human cognitive ability. Neurosci Lett 438:163-167.

Garwood J, Heck N, Reichardt F, Faissner A (2003) Phosphacan short isoform, a novel non-proteoglycan variant of phosphacan/receptor protein tyrosine phosphatase-beta, interacts with neuronal receptors and promotes neurite outgrowth. J Biol Chem 278:24164-24173.

Glaser EM, Van der Loos H (1981) Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. J Neurosci Methods 4:117-125.

Goldberg JL (2004) Intrinsic neuronal regulation of axon and dendrite growth. Curr Opin Neurobiol 14:551-557.

Gonzalez-Brito MR, Bixby JL (2006) Differential activities in adhesion and neurite growth of fibronectin type III repeats in the PTP-delta extracellular domain. Int J Dev Neurosci 24:425-429.

Govek EE, Newey SE, Akerman CJ, Cross JR, Van der Veken L, Van Aelst L (2004) The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. Nat Neurosci 7:364-372.

Grabrucker AM, Knight MJ, Proepper C, Bockmann J, Joubert M, Rowan M, Nienhaus GU, Garner CC, Bowie JU, Kreutz MR, Gundelfinger ED, Boeckers TM Concerted action of zinc and ProSAP/Shank in synaptogenesis and synapse maturation. Embo J 30:569-581.

Granger AJ, Shi Y, Lu W, Cerpas M, Nicoll RA LTP requires a reserve pool of glutamate receptors independent of subunit type. Nature 493:495-500.

Greenberg ME, Xu B, Lu B, Hempstead BL (2009) New insights in the biology of BDNF synthesis and release: implications in CNS function. J Neurosci 29:12764-12767.

Gregory KJ, Dong EN, Meiler J, Conn PJ Allosteric modulation of metabotropic glutamate receptors: structural insights and therapeutic potential. Neuropharmacology 60:66-81.

Grueber WB, Ye B, Moore AW, Jan LY, Jan YN (2003) Dendrites of distinct classes of Drosophila sensory neurons show different capacities for homotypic repulsion. Curr Biol 13:618-626.

Grutzendler J, Kasthuri N, Gan WB (2002) Long-term dendritic spine stability in the adult cortex. Nature 420:812-816.

Han JM, Sahin M TSC1/TSC2 signaling in the CNS. FEBS Lett 585:973-980.

Hand R, Bortone D, Mattar P, Nguyen L, Heng JI, Guerrier S, Boutt E, Peters E, Barnes AP, Parras C, Schuurmans C, Guillemot F, Polleux F (2005) Phosphorylation of Neurogenin2 specifies the migration properties and the dendritic morphology of pyramidal neurons in the neocortex. Neuron 48:45-62.

Harris KM (1999) Structure, development, and plasticity of dendritic spines. Curr Opin Neurobiol 9:343-348.

Hayashi T, Yoshida T, Ra M, Taguchi R, Mishina M IL1RAPL1 associated with mental retardation and autism regulates the formation and stabilization of glutamatergic synapses of cortical neurons through RhoA signaling pathway. PLoS One 8:e66254.

Hendriks WJ, Pulido R Protein tyrosine phosphatase variants in human hereditary disorders and disease susceptibilities. Biochim Biophys Acta 1832:1673-1696.

Hollander E Social synchrony and oxytocin: from behavior to genes to therapeutics. Am J Psychiatry 170:1086-1089.

Hollander E, Novotny S, Hanratty M, Yaffe R, DeCaria CM, Aronowitz BR, Mosovich S (2003) Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. Neuropsychopharmacology 28:193-198.
Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, Soorya L, Anagnostou E, Wasserman S (2007) Oxytocin increases retention of social cognition in autism. Biol Psychiatry 61:498-503.
Holtmaat A, Svoboda K (2009) Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev Neurosci 10:647-658.
Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, Svoboda K (2005) Transient and persistent dendritic spines in the neocortex in vivo. Neuron 45:279-291.
Houbaert X, Zhang CL, Gambino F, Lepleux M, Deshors M, Normand E, Levet F, Ramos M, Billuart P, Chelly J, Herzog E, Humeau Y Target-specific vulnerability of excitatory synapses leads to deficits in associative memory in a model of intellectual disorder. J Neurosci 33:13805-13819.
Huttner WB, Schiebler W, Greengard P, De Camilli P (1983) Synapsin I (protein I), a nerve terminal-specific phosphoprotein. III. Its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation. J Cell Biol 96:1374-1388.
Inglis FM, Zuckerman KE, Kalb RG (2000) Experience-dependent development of spinal motor neurons. Neuron 26:299-305.
Jaffe AB, Hall A (2005) Rho GTPases: biochemistry and biology. Annu Rev Cell Dev Biol 21:247-269.
Jan YN, Jan LY Branching out: mechanisms of dendritic arborization. Nat Rev Neurosci 11:316-328.
Jaworski J, Spangler S, Seeburg DP, Hoogenraad CC, Sheng M (2005) Control of dendritic arborization by the phosphoinositide-3'-kinase-Akt-mammalian target of rapamycin pathway. J Neurosci 25:11300-11312.
Jossin Y, Goffinet AM (2007) Reelin signals through phosphatidylinositol 3-kinase and Akt to control cortical development and through mTor to regulate dendritic growth. Mol Cell Biol 27:7113-7124.
Kanaani J, el-Husseini Ael D, Aguilera-Moreno A, Diacovo JM, Bredt DS, Baekkeskov S (2002) A combination of three distinct trafficking signals mediates axonal targeting and presynaptic clustering of GAD65. J Cell Biol 158:1229-1238.
Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133-1138.
Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, Chen TB, Wisnoski DD, Lindsley CW, Tiller PR, Smith S, Jacobson MA, Sur C, Duggan ME, Pettibone DJ, Conn PJ, Williams DL, Jr. (2005) A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has in vivo activity and antipsychotic-like effects in rat behavioral models. J Pharmacol Exp Ther 313:199-206.
Koh JW, Kang SY, Kim GH, Yoo HW, Yu J Central precocious puberty in a patient with X-linked adrenal hypoplasia congenita and Xp21 contiguous gene deletion syndrome. Ann Pediatr Endocrinol Metab 18:90-94.
Kolomeets NS, Orlovskaya DD, Uranova NA (2007) Decreased numerical density of CA3 hippocampal mossy fiber synapses in schizophrenia. Synapse 61:615-621.
Kosaka H, Munesue T, Ishitobi M, Asano M, Omori M, Sato M, Tomoda A, Wada Y Long-term oxytocin administration improves social behaviors in a girl with autistic disorder. BMC Psychiatry 12:110.
Kreutz MR, Sala C Synaptic plasticity dynamics, development and disease. In, pp 1 online resource (ix, 618 p.). Vienna ; New York: Springer.
Krueger DD, Bear MF Toward fulfilling the promise of molecular medicine in fragile X syndrome. Annu Rev Med 62:411-429.
Kulkarni VA, Firestein BL The dendritic tree and brain disorders. Mol Cell Neurosci 50:10-20.
Kumar V, Zhang MX, Swank MW, Kunz J, Wu GY (2005) Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. J Neurosci 25:11288-11299.
Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF (2006) Pten regulates neuronal arborization and social interaction in mice. Neuron 50:377-388.
Kwon SE, Chapman ER Synaptophysin regulates the kinetics of synaptic vesicle endocytosis in central neurons. Neuron 70:847-854.
Kwon SK, Woo J, Kim SY, Kim H, Kim E Trans-synaptic adhesions between netrin-G ligand-3 (NGL-3) and receptor tyrosine phosphatases LAR, protein-tyrosine phosphatase delta (PTPdelta), and PTPsigma via specific domains regulate excitatory synapse formation. J Biol Chem 285:13966-13978.
Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
Law AJ, Weickert CS, Hyde TM, Kleinman JE, Harrison PJ (2004) Reduced spinophilin but not microtubule-associated protein 2 expression in the hippocampal formation in schizophrenia and mood disorders: molecular evidence for a pathology of dendritic spines. Am J Psychiatry 161:1848-1855.
Lee S, Stevens CF (2007) General design principle for scalable neural circuits in a vertebrate retina. Proc Natl Acad Sci U S A 104:12931-12935.
Leemhuis J, Boutillier S, Barth H, Feuerstein TJ, Brock C, Nurnberg B, Aktories K, Meyer DK (2004) Rho GTPases and phosphoinositide 3-kinase organize formation of branched dendrites. J Biol Chem 279:585-596.

Lehrke R (1972) Theory of X-linkage of major intellectual traits. Am J Ment Defic 76:611-619.
Lepretre F, Delannoy V, Froguel P, Vasseur F, Montpellier C (2003) Dissection of an inverted X(p21.3q27.1) chromosome associated with mental retardation. Cytogenet Genome Res 101:124-129.
Li Z, Sheng M (2003) Some assembly required: the development of neuronal synapses. Nat Rev Mol Cell Biol 4:833-841.
Lin YC, Koleske AJ Mechanisms of synapse and dendrite maintenance and their disruption in psychiatric and neurodegenerative disorders. Annu Rev Neurosci 33:349-378.
Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl) 92:180-185.
Liu X, Kawamura Y, Shimada T, Otowa T, Koishi S, Sugiyama T, Nishida H, Hashimoto O, Nakagami R, Tochigi M, Umekage T, Kano Y, Miyagawa T, Kato N, Tokunaga K, Sasaki T Association of the oxytocin receptor (OXTR) gene polymorphisms with autism spectrum disorder (ASD) in the Japanese population. J Hum Genet 55:137-141.
Lodish HF (2000) Molecular cell biology, 4th Edition. New York: W.H. Freeman.
Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. Nature 452:436-441.
MacNeil MA, Masland RH (1998) Extreme diversity among amacrine cells: implications for function. Neuron 20:971-982.
Majewska AK, Newton JR, Sur M (2006) Remodeling of synaptic structure in sensory cortical areas in vivo. J Neurosci 26:3021-3029.
Malhotra D, McCarthy S, Michaelson JJ, Vacic V, Burdick KE, Yoon S, Cichon S, Corvin A, Gary S, Gershon ES, Gill M, Karayiorgou M, Kelsoe JR, Krastoshevsky O, Krause V, Leibenluft E, Levy DL, Makarov V, Bhandari A, Malhotra AK, McMahon FJ, Nothen MM, Potash JB, Rietschel M, Schulze TG, Sebat J High frequencies of de novo CNVs in bipolar disorder and schizophrenia. Neuron 72:951-963.
Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, Chen G, Gage FH, Muotri AR A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell 143:527-539.
McAllister AK (2000) Cellular and molecular mechanisms of dendrite growth. Cereb Cortex 10:963-973.
McAllister AK (2002) Neurotrophins and cortical development. Results Probl Cell Differ 39:89-112.
McPheeters ML, Warren Z, Sathe N, Bruzek JL, Krishnaswami S, Jerome RN, Veenstra-Vanderweele J A systematic review of medical treatments for children with autism spectrum disorders. Pediatrics 127:e1312-1321.
Meijering E, Jacob M, Sarria JC, Steiner P, Hirling H, Unser M (2004) Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. Cytometry A 58:167-176.
Mignon-Ravix C, Cacciagli P, Choucair N, Popovici C, Missirian C, Milh M, Megarbane A, Busa T, Julia S, Girard N, Badens C, Sigaudy S, Philip N, Villard L Intragenic rearrangements in X-linked intellectual deficiency: results of a-CGH in a series of 54 patients and identification of TRPC5 and KLHL15 as potential XLID genes. Am J Med Genet A 164A:1991-1997.
Milev P, Friedlander DR, Sakurai T, Karthikeyan L, Flad M, Margolis RK, Grumet M, Margolis RU (1994) Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia, and neural cell adhesion molecules. J Cell Biol 127:1703-1715.
Miller FD, Kaplan DR (2003) Signaling mechanisms underlying dendrite formation. Curr Opin Neurobiol 13:391-398.
Milner LD, Landmesser LT (1999) Cholinergic and GABAergic inputs drive patterned spontaneous motoneuron activity before target contact. J Neurosci 19:3007-3022.
Mineur YS, Sluyter F, de Wit S, Oostra BA, Crusio WE (2002) Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse. Hippocampus 12:39-46.
Mizuno K, Hasegawa K, Katagiri T, Ogimoto M, Ichikawa T, Yakura H (1993) MPTP delta, a putative murine homolog of HPTP delta, is expressed in specialized regions of the brain and in the B-cell lineage. Mol Cell Biol 13:5513-5523.
Nakatani J, Tamada K, Hatanaka F, Ise S, Ohta H, Inoue K, Tomonaga S, Watanabe Y, Chung YJ, Banerjee R, Iwamoto K, Kato T, Okazawa M, Yamauchi K, Tanda K, Takao K, Miyakawa T, Bradley A, Takumi T (2009) Abnormal behavior in a chromosome-engineered mouse model for human 15q11-13 duplication seen in autism. Cell 137:1235-1246.
Nawara M, Klapecki J, Borg K, Jurek M, Moreno S, Tryfon J, Bal J, Chelly J, Mazurczak T (2008) Novel mutation of IL1RAPL1 gene in a nonspecific X-linked mental retardation (MRX) family. Am J Med Genet A 146A:3167-3172.
Newey SE, Velamoor V, Govek EE, Van Aelst L (2005) Rho GTPases, dendritic structure, and mental retardation. J Neurobiol 64:58-74.
Ogier M, Wang H, Hong E, Wang Q, Greenberg ME, Katz DM (2007) Brain-derived neurotrophic factor expression and respiratory function improve after ampakine treatment in a mouse model of Rett syndrome. J Neurosci 27:10912-10917.
O'Kusky JR, Ye P, D'Ercole AJ (2000) Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. J Neurosci 20:8435-8442.

Ozdinler PH, Macklis JD (2006) IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. Nat Neurosci 9:1371-1381.

Pardo CA, Eberhart CG (2007) The neurobiology of autism. Brain Pathol 17:434-447.

Parrish JZ, Emoto K, Kim MD, Jan YN (2007) Mechanisms that regulate establishment, maintenance, and remodeling of dendritic fields. Annu Rev Neurosci 30:399-423.

Parrish JZ, Xu P, Kim CC, Jan LY, Jan YN (2009) The microRNA bantam functions in epithelial cells to regulate scaling growth of dendrite arbors in drosophila sensory neurons. Neuron 63:788-802.

Pavlowsky A, Gianfelice A, Pallotto M, Zanchi A, Vara H, Khelfaoui M, Valnegri P, Rezai X, Bassani S, Brambilla D, Kumpost J, Blahos J, Roux MJ, Humeau Y, Chelly J, Passafaro M, Giustetto M, Billuart P, Sala C A postsynaptic signaling pathway that may account for the cognitive defect due to IL1RAPL1 mutation. Curr Biol 20:103-115.

Peier AM, McIlwain KL, Kenneson A, Warren ST, Paylor R, Nelson DL (2000) (Over)correction of FMR1 deficiency with YAC transgenics: behavioral and physical features. Hum Mol Genet 9:1145-1159.

Picker JD, Walsh CA New innovations: therapeutic opportunities for intellectual disabilities. Ann Neurol 74:382-390.

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bolte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BH, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466:368-372.

Piton A, Michaud JL, Peng H, Aradhya S, Gauthier J, Mottron L, Champagne N, Lafreniere RG, Hamdan FF, Joober R, Fombonne E, Marineau C, Cossette P, Dube MP, Haghighi P, Drapeau P, Barker PA, Carbonetto S, Rouleau GA (2008) Mutations in the calcium-related gene IL1RAPL1 are associated with autism. Hum Mol Genet 17:3965-3974.

Portera-Cailliau C, Pan DT, Yuste R (2003) Activity-regulated dynamic behavior of early dendritic protrusions: evidence for different types of dendritic filopodia. J Neurosci 23:7129-7142.

Posey DJ, Kem DL, Swiezy NB, Sweeten TL, Wiegand RE, McDougle CJ (2004) A pilot study of D-cycloserine in subjects with autistic disorder. Am J Psychiatry 161:2115-2117.

Pulido R, Serra-Pages C, Tang M, Streuli M (1995) The LAR/PTP delta/PTP sigma subfamily of transmembrane protein-tyrosine-phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP.1. Proc Natl Acad Sci U S A 92:11686-11690.

Purves D (2008) Neuroscience, 4th Edition. Sunderland, Mass.: Sinauer.

Radons J, Dove S, Neumann D, Altmann R, Botzki A, Martin MU, Falk W (2003) The interleukin 1 (IL-1) receptor accessory protein Toll/IL-1 receptor domain: analysis of putative interaction sites in vitro mutagenesis and molecular modeling. J Biol Chem 278:49145-49153.

Ramocki MB, Zoghbi HY (2008) Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. Nature 455:912-918.

Ramos-Brossier M, Montani C, Lebrun N, Gritti L, Martin C, Seminatore-Nole C, Toussaint A, Moreno S, Poirier K, Dorseuil O, Chelly J, Hackett A, Gecz J, Bieth E, Faudet A, Heron D, Kooy RF, Loeys B, Humeau Y, Sala C, Billuart P Novel IL1RAPL1 mutations associated with intellectual disability impair synaptogenesis. Hum Mol Genet.

Rocha M, Sur M (1995) Rapid acquisition of dendritic spines by visual thalamic neurons after blockade of N-methyl-D-aspartate receptors. Proc Natl Acad Sci U S A 92:8026-8030.

Rolls MM, Satoh D, Clyne PJ, Henner AL, Uemura T, Doe CQ (2007) Polarity and intracellular compartmentalization of Drosophila neurons. Neural Dev 2:7.

Rudolph U, Knoflach F Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. Nat Rev Drug Discov 10:685-697.

Sajnani G, Aricescu AR, Jones EY, Gallagher J, Alete D, Stoker A (2005) PTPsigma promotes retinal neurite outgrowth non-cell-autonomously. J Neurobiol 65:59-71.

Sala C, Segal M Dendritic spines: the locus of structural and functional plasticity. Physiol Rev 94:141-188.

Sala C, Piech V, Wilson NR, Passafaro M, Liu G, Sheng M (2001) Regulation of dendritic spine morphology and synaptic function by Shank and Homer. Neuron 31:115-130.

Sanes DH, Markowitz S, Bernstein J, Wardlow J (1992) The influence of inhibitory afferents on the development of postsynaptic dendritic arbors. J Comp Neurol 321:637-644.
Sarto I, Wabnegger L, Dogl E, Sieghart W (2002) Homologous sites of GABA(A) receptor alpha(1), beta(3) and gamma(2) subunits are important for assembly. Neuropharmacology 43:482-491.
Satoh D, Sato D, Tsuyama T, Saito M, Ohkura H, Rolls MM, Ishikawa F, Uemura T (2008) Spatial control of branching within dendritic arbors by dynein-dependent transport of Rab5-endosomes. Nat Cell Biol 10:1164-1171.
Schaapveld RQ, Schepens JT, Bachner D, Attema J, Wieringa B, Jap PH, Hendriks WJ (1998) Developmental expression of the cell adhesion molecule-like protein tyrosine phosphatases LAR, RPTPdelta and RPTPsigma in the mouse. Mech Dev 77:59-62.
Schormair B, Kemlink D, Roeske D, Eckstein G, Xiong L, Lichtner P, Ripke S, Trenkwalder C, Zimprich A, Stiasny-Kolster K, Oertel W, Bachmann CG, Paulus W, Hogl B, Frauscher B, Gschliesser V, Poewe W, Peglau I, Vodicka P, Vavrova J, Sonka K, Nevsimalova S, Montplaisir J, Turecki G, Rouleau G, Gieger C, Illig T, Wichmann HE, Holsboer F, Muller-Myhsok B, Meitinger T, Winkelmann J (2008) PTPRD (protein tyrosine phosphatase receptor type delta) is associated with restless legs syndrome. Nat Genet 40:946-948.
Scott EK, Luo L (2001) How do dendrites take their shape? Nat Neurosci 4:359-365.
Sfakianos MK, Eisman A, Gourley SL, Bradley WD, Scheetz AJ, Settleman J, Taylor JR, Greer CA, Williamson A, Koleske AJ (2007) Inhibition of Rho via Arg and p190RhoGAP in the postnatal mouse hippocampus regulates dendritic spine maturation, synapse and dendrite stability, and behavior. J Neurosci 27:10982-10992.
Shatz CJ (1996) Emergence of order in visual system development. Proc Natl Acad Sci U S A 93:602-608.
Sheng M, Kim E The postsynaptic organization of synapses. Cold Spring Harb Perspect Biol 3.
Sheng M, Kim MJ (2002) Postsynaptic signaling and plasticity mechanisms. Science 298:776-780.
Sheng M, Hoogenraad CC (2007) The postsynaptic architecture of excitatory synapses: a more quantitative view. Annu Rev Biochem 76:823-847.
Solomon DA, Kim JS, Cronin JC, Sibenaller Z, Ryken T, Rosenberg SA, Ressom H, Jean W, Bigner D, Yan H, Samuels Y, Waldman T (2008) Mutational inactivation of PTPRD in glioblastoma multiforme and malignant melanoma. Cancer Res 68:10300-10306.
Spooren W, Lindemann L, Ghosh A, Santarelli L Synapse dysfunction in autism: a molecular medicine approach to drug discovery in neurodevelopmental disorders. Trends Pharmacol Sci 33:669-684.
Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic integration. Nat Rev Neurosci 9:206-221.
Stefani MR, Moghaddam B Activation of type 5 metabotropic glutamate receptors attenuates deficits in cognitive flexibility induced by NMDA receptor blockade. Eur J Pharmacol 639:26-32.
Stockmeier CA, Mahajan GJ, Konick LC, Overholser JC, Jurjus GJ, Meltzer HY, Uylings HB, Friedman L, Rajkowska G (2004) Cellular changes in the postmortem hippocampus in major depression. Biol Psychiatry 56:640-650.
Sudhof TC (2004) The synaptic vesicle cycle. Annu Rev Neurosci 27:509-547.
Sudhof TC (2008) Neuroligins and neurexins link synaptic function to cognitive disease. Nature 455:903-911.
Sudhof TC, Rizo J Synaptic vesicle exocytosis. Cold Spring Harb Perspect Biol 3.
Tabolacci E, Pomponi MG, Pietrobono R, Terracciano A, Chiurazzi P, Neri G (2006) A truncating mutation in the IL1RAPL1 gene is responsible for X-linked mental retardation in the MRX21 family. Am J Med Genet A 140:482-487.
Tabuchi K, Biederer T, Butz S, Sudhof TC (2002) CASK participates in alternative tripartite complexes in which Mint 1 competes for binding with caskin 1, a novel CASK-binding protein. J Neurosci 22:4264-4273.
Tada T, Sheng M (2006) Molecular mechanisms of dendritic spine morphogenesis. Curr Opin Neurobiol 16:95-101.
Takagi N, Sugimoto M, Yamaguchi S, Ito M, Tan SS, Okabe M (2002) Nonrandom X chromosome inactivation in mouse embryos carrying Searle's T(X;16)16H translocation visualized using X-linked LACZ and GFP transgenes. Cytogenet Genome Res 99:52-58.
Takahashi H, Craig AM Protein tyrosine phosphatases PTPdelta, PTPsigma, and LAR: presynaptic hubs for synapse organization. Trends Neurosci 36:522-534.
Takahashi H, Katayama K, Sohya K, Miyamoto H, Prasad T, Matsumoto Y, Ota M, Yasuda H, Tsumoto T, Aruga J, Craig AM Selective control of inhibitory synapse development by Slitrk3-PTPdelta trans-synaptic interaction. Nat Neurosci 15:389-398, S381-382.
Tamminga CA, Southcott S, Sacco C, Wagner AD, Ghose S Glutamate dysfunction in hippocampus: relevance of dentate gyrus and CA3 signaling. Schizophr Bull 38:927-935.
Tarpey PS, Smith R, Pleasance E, Whibley A, Edkins S, Hardy C, O'Meara S, Latimer C, Dicks E, Menzies A, Stephens P, Blow M, Greenman C, Xue Y, Tyler-Smith C, Thompson D, Gray K, Andrews J, Barthorpe S, Buck G, Cole J, Dunmore R, Jones D, Maddison M, Mironenko T, Turner R, Turrell K, Varian J, West S, Widaa S, Wray P, Teague J, Butler A, Jenkinson A, Jia M, Richardson D, Shepherd R, Wooster R, Tejada MI, Martinez F, Carvill G, Goliath R, de Brouwer AP, van Bokhoven H, Van Esch H, Chelly J, Raynaud M, Ropers HH, Abidi FE, Srivastava AK, Cox J, Luo Y, Mallya U, Moon J, Parnau J, Mohammed S, Tolmie JL, Shoubridge C, Corbett M, Gardner A, Haan E, Rujirabanjerd S, Shaw M, Vandeleur L, Fullston T, Easton DF,

Boyle J, Partington M, Hackett A, Field M, Skinner C, Stevenson RE, Bobrow M, Turner G, Schwartz CE, Gecz J, Raymond FL, Futreal PA, Stratton MR (2009) A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. Nat Genet 41:535-543.

Tropea A, Miceli F, Minici F, Tiberi F, Orlando M, Gangale MF, Romani F, Catino S, Mancuso S, Navarra P, Lanzone A, Apa R (2006) Regulation of vascular endothelial growth factor synthesis and release by human luteal cells in vitro. J Clin Endocrinol Metab 91:2303-2309.

Tsai PT, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, Steinberg J, Crawley JN, Regehr WG, Sahin M Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. Nature 488:647-651.

Uetani N, Kato K, Ogura H, Mizuno K, Kawano K, Mikoshiba K, Yakura H, Asano M, Iwakura Y (2000) Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice. Embo J 19:2775-2785.

Um JW, Ko J LAR-RPTPs: synaptic adhesion molecules that shape synapse development. Trends Cell Biol 23:465-475.

Urbanska M, Blazejczyk M, Jaworski J (2008) Molecular basis of dendritic arborization. Acta Neurobiol Exp (Wars) 68:264-288.

Uslaner JM, Smith SM, Huszar SL, Pachmerhiwala R, Hinchliffe RM, Vardigan JD, Hutson PH (2009) Combined administration of an mGlu2/3 receptor agonist and a 5-HT 2A receptor antagonist markedly attenuate the psychomotor-activating and neurochemical effects of psychostimulants. Psychopharmacology (Berl) 206:641-651.

Valnegri P, Montrasio C, Brambilla D, Ko J, Passafaro M, Sala C The X-linked intellectual disability protein IL1RAPL1 regulates excitatory synapse formation by binding PTPdelta and RhoGAP2. Hum Mol Genet 20:4797-4809.

Verpelli C, Montani C, Vicidomini C, Heise C, Sala C Mutations of the synapse genes and intellectual disability syndromes. Eur J Pharmacol 719:112-116.

Verpelli C, Piccoli G, Zibetti C, Zanchi A, Gardoni F, Huang K, Brambilla D, Di Luca M, Battaglioli E, Sala C Synaptic activity controls dendritic spine morphology by modulating eEF2-dependent BDNF synthesis. J Neurosci 30:5830-5842.

Verpelli C, Dvoretskova E, Vicidomini C, Rossi F, Chiappalone M, Schoen M, Di Stefano B, Mantegazza R, Broccoli V, Bockers TM, Dityatev A, Sala C Importance of Shank3 protein in regulating metabotropic glutamate receptor 5 (mGluR5) expression and signaling at synapses. J Biol Chem 286:34839-34850.

Vessey JP, Macchi P, Stein JM, Mikl M, Hawker KN, Vogelsang P, Wieczorek K, Vendra G, Riefler J, Tubing F, Aparicio SA, Abel T, Kiebler MA (2008) A loss of function allele for murine Staufen1 leads to impairment of dendritic Staufen1-RNP delivery and dendritic spine morphogenesis. Proc Natl Acad Sci U S A 105:16374-16379.

von Bohlen Und Halbach O Dendritic spine abnormalities in mental retardation. Cell Tissue Res 342:317-323.

Wallace MJ, Fladd C, Batt J, Rotin D (1998) The second catalytic domain of protein tyrosine phosphatase delta (PTP delta) binds to and inhibits the first catalytic domain of PTP sigma. Mol Cell Biol 18:2608-2616.

Walsh CA, Morrow EM, Rubenstein JL (2008) Autism and brain development. Cell 135:396-400.

Wang J, Bixby JL (1999) Receptor tyrosine phosphatase-delta is a homophilic, neurite-promoting cell adhesion molecular for CNS neurons. Mol Cell Neurosci 14:370-384.

Wassle H, Boycott BB (1991) Functional architecture of the mammalian retina. Physiol Rev 71:447-480.

Welch JM, Lu J, Rodriguiz RM, Trotta NC, Peca J, Ding JD, Feliciano C, Chen M, Adams JP, Luo J, Dudek SM, Weinberg RJ, Calakos N, Wetsel WC, Feng G (2007) Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. Nature 448:894-900.

Wheway JM, Yau SC, Nihalani V, Ellis D, Irving M, Splitt M, Roberts RG (2003) A complex deletion-inversion-deletion event results in a chimeric IL1RAPL1-dystrophin transcript and a contiguous gene deletion syndrome. J Med Genet 40:127-131.

Williams DW, Truman JW (2004) Mechanisms of dendritic elaboration of sensory neurons in Drosophila: insights from in vivo time lapse. J Neurosci 24:1541-1550.

Won H, Mah W, Kim E Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses. Front Mol Neurosci 6:19.

Won H, Lee HR, Gee HY, Mah W, Kim JI, Lee J, Ha S, Chung C, Jung ES, Cho YS, Park SG, Lee JS, Lee K, Kim D, Bae YC, Kaang BK, Lee MG, Kim E Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. Nature 486:261-265.

Woodings JA, Sharp SJ, Machesky LM (2003) MIM-B, a putative metastasis suppressor protein, binds to actin and to protein tyrosine phosphatase delta. Biochem J 371:463-471.

Wu GY, Zou DJ, Rajan I, Cline H (1999) Dendritic dynamics in vivo change during neuronal maturation. J Neurosci 19:4472-4483.

Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP (2005) Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 49:1053-1066.

Yang T, Yin W, Derevyanny VD, Moore LA, Longo FM (2005) Identification of an ectodomain within the LAR protein tyrosine phosphatase receptor that binds homophilically and activates signalling pathways promoting neurite outgrowth. Eur J Neurosci 22:2159-2170.

Yasumura M, Yoshida T, Yamazaki M, Abe M, Natsume R, Kanno K, Uemura T, Takao K, Sakimura K, Kikusui T, Miyakawa T, Mishina M IL1RAPL1 knockout mice show spine density decrease, learning deficiency, hyperactivity and reduced anxiety-like behaviours. Sci Rep 4:6613.

Ye B, Petritsch C, Clark IE, Gavis ER, Jan LY, Jan YN (2004) Nanos and Pumilio are essential for dendrite morphogenesis in Drosophila peripheral neurons. Curr Biol 14:314-321.

Yoshida T, Mishina M (2008) Zebrafish orthologue of mental retardation protein IL1RAPL1 regulates presynaptic differentiation. Mol Cell Neurosci 39:218-228.

Yoshida T, Yasumura M, Uemura T, Lee SJ, Ra M, Taguchi R, Iwakura Y, Mishina M IL-1 receptor accessory protein-like 1 associated with mental retardation and autism mediates synapse formation by trans-synaptic interaction with protein tyrosine phosphatase delta. J Neurosci 31:13485-13499.

Yoshida T, Shiroshima T, Lee SJ, Yasumura M, Uemura T, Chen X, Iwakura Y, Mishina M Interleukin-1 receptor accessory protein organizes neuronal synaptogenesis as a cell adhesion molecule. J Neurosci 32:2588-2600.

Youngs EL, Henkhaus R, Hellings JA, Butler MG IL1RAPL1 gene deletion as a cause of X-linked intellectual disability and dysmorphic features. Eur J Med Genet 55:32-36.

Yrigollen CM, Han SS, Kochetkova A, Babitz T, Chang JT, Volkmar FR, Leckman JF, Grigorenko EL (2008) Genes controlling affiliative behavior as candidate genes for autism. Biol Psychiatry 63:911-916.

Yuste R, Bonhoeffer T (2004) Genesis of dendritic spines: insights from ultrastructural and imaging studies. Nat Rev Neurosci 5:24-34.

Yuste R, Nelson DA, Rubin WW, Katz LC (1995) Neuronal domains in developing neocortex: mechanisms of coactivation. Neuron 14:7-17.

Zhang CL, Houbaert X, Lepleux M, Deshors M, Normand E, Gambino F, Herzog E, Humeau Y The hippocampo-amygdala control of contextual fear expression is affected in a model of intellectual disability. Brain Struct Funct.

Zheng Y, Wildonger J, Ye B, Zhang Y, Kita A, Younger SH, Zimmerman S, Jan LY, Jan YN (2008) Dynein is required for polarized dendritic transport and uniform microtubule orientation in axons. Nat Cell Biol 10:1172-1180.

Zuchner S, Wendland JR, Ashley-Koch AE, Collins AL, Tran-Viet KN, Quinn K, Timpano KC, Cuccaro ML, Pericak-Vance MA, Steffens DC, Krishnan KR, Feng G, Murphy DL (2009) Multiple rare SAPAP3 missense variants in trichotillomania and OCD. Mol Psychiatry 14:6-9.