Brainconf – Synaptic Plasticity

Posters

Group A:
- Wednesday during lunch break
- Wednesday during Wine and cheese

Group B :
- Thursday during lunch break
- Friday during lunch

By family name

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Baginska – A2
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By poster number

Group A

Group B

A1 - Tracking the synaptic alterations in a knock-in mouse model of Alzheimer's disease

Luis Enrique Arroyo-García[1], Giusy Pizzirusso[1,4], Julen Goikolea[2], Maria Latorre-Leal[2], Efthalia Preka[4], Silvia Maioli[2], Per Nilsson[3], Adamantia Fragkopoulou [4], Klas Blomgren[4] and André Fisahn[1].

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Our goal is to investigate the synaptic alterations that lead to brain oscillatory activity impairment in Alzheimer's disease (AD). To do so, we use a knock-in mouse model of AD (App^{NL-G-F}) and evaluate gamma oscillations ex vivo. Using this model, we have demonstrated that the hippocampal oscillatory activity in the gamma band is strongly impaired before the onset of amyloid-beta (Aβ) plaques. However, the exact breaking point during the amyloidogenic progression in the App^{NL-G-F} model is still unknown. Here, we aim to track the breaking point and the molecular pathways that drive the hippocampal gamma oscillations impairment in the App^{NL-G-F} model.

We used *in vitro* electrophysiological techniques to analyze the circuitry involving gamma oscillations, inhibitory interneurons, and excitatory pyramidal cells in the hippocampal network from 1 to 2 months of age. Moreover, we performed hippocampal bulk RNA-sequencing and western-blot (WB) analysis at 1 and 2 months of age in WT and App^{NL-G-F} mice.

In vitro-gamma oscillations degrade progressively and become significantly impaired at postnatal day (PD) 50. Spike-gamma coupling from inhibitory interneurons is impaired after PD 50. Moreover, we identified with the RNA-seq analysis that the excitatory and inhibitory pathways worsen with the progression of the pathology in the App^{NL-G-F} mice, and we confirmed by WB.

Our results demonstrate that the amyloidogenic progression in the App^{NL-G-F} model causes a synaptic collapse that affects the cognitive-relevant hippocampal gamma oscillations before the onset of Aβ plaques. These results provide a time window to explore the pathological synaptic modifications in the hippocampal network.

A2 - The replenishment of dense core vesicles pool in mammalian CNS neurons

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2 Clinical Genetics, VU Medical Center, Amsterdam, The Netherlands

The human brain processes information through the release of neurotransmitters and neuropeptides. Neurotransmitters are stored in synaptic vesicles (SVs), whereas neuropeptides are carried by dense core vesicles (DCVs). The release of neurotransmitters relies of low frequency electric stimulation, while

the release of neuropeptides requires high frequency or patterned electric stimulation. Here, we systematically studied fusion of DCVs upon the electric field stimulation using fluorescent DCV reporters in mammalian CNS neurons. We showed that high tetanic stimulation consisting of multiple trains of action potentials delivered at 50 Hz frequency was the most efficient stimulation for DCV fusion and leaded to the depletion of the DCV releasable pool. The introduction of inter-burst intervals in the tetanic stimulation did not increase DCV fusion, but it allowed the DCV fusogenic pool to be restored. Theta burst stimulation (TBS) triggered less DCV fusion than tetanic stimulation, but its effectiveness depended on the number of applied action potentials. Low frequency stimulation (4 Hz) intermitted with fast ripple stimulation (200 Hz) did not potentiate the fusion of DCVs. Lastly, we showed that stimulation paradigms influence the properties of individual fusion events, promoting longer DCV fusion pore openings with more intense electrical stimulation. Thus, different frequency patterns triggered DCV exocytosis differently, based on the number of action potentials and their frequency.

A3 - Multimodal profiling of developing Purkinje cells using Patch-Seq

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During the first postnatal weeks, each initially multi-innervated Purkinje cell (PC) in the cerebellum cortex selects a single climbing fiber (CF) through an activity-dependent competition process: the most active CF will be its only input from the inferior olive (Hashimoto *et al*., *Neuron* 2009). Meanwhile, weak CFs synapses are eliminated through LTD-like mechanisms (Piochon *et al., Nat Naurosci* 2016). Nevertheless, how this single CF afferent is stabilized and maintained over the individual's life remains unknown. We hypothesize that selected circuits are molecularly encoded during developmental critical periods, possibly through the expression of certain cell adhesion molecules (CAMs) able to specify and stabilize the strongest connections. Among them, protocadherins (Pcdh) are attractive candidates due to their combinatorial expression pattern across individual PCs, a key feature for which they have been proposed to encode PCs individuality, thus possibly contributing to synaptic specificity (Sanes and Zipursky, *Cell* 2020). To study which CAM families may be involved in this process, we implemented Patch-Seq in cerebellar acute slices obtained at key postnatal timepoints (i.e., P5, P10 and P21) covering multi-innervation to mono-innervation progression. Patch-Seq provides electrophysiology, morphology and transcriptomic data for single PCs, hence enabling direct correlation between synaptic competition, dendritic tree development and gene expression for individual neurons. Our preliminary data suggests a decrease in Pcdhs isoforms diversity correlated with changes in CF innervation during this developmental critical period. Therefore, long-term molecular encoding of CF-PC circuit may rely on Pcdh isoforms variation, as well as other CAM families.

A4 - Study of Synaptic Plasticity Mechanisms Underlying Memory Consolidation in the Hippocampo-Neocortical Network

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Learning and consolidation of new behavioural adaptations is a multistep process, taking place over multiple days, which implicates an active communication between the hippocampus and associative cortical areas. Consolidation has been causally related to Sharp Wave-Ripples (SW-Rs) events taking place in the hippocampus during Slow Wave Sleep and driving replays of wakeful-acquired memory-related assemblies in both hippocampus and cortices. SW-Rs are thought to strengthen synaptic connections within memory-related assemblies via synaptic plasticity mechanisms, however direct proof of this concept has been hard to obtain. The aim of my research is to unravel the synaptic mechanism underlying hippocampal to associative cortical areas communication during memory consolidation and address whether and when synaptic plasticity takes place at hippocampal to cortical synapses. To this aim, I record local field potentials and unitary activities from extracellular electrodes placed in the dorsal hippocampus, medial prefrontal (mPFC) and posterior parietal (PPC) cortices in freely behaving animals performing a spatial memory task and during a 3 hours resting period following the task. Both cortices are implicated in decision making and cognitive control, but differ in their anatomical connection to the hippocampus and in their specific cognitive function. My results show that neuronal firing of excitatory pyramidal cells and inhibitory interneurons in the mPFC during the occurrence of hippocampal SW-Rs is enhanced after learning, suggesting a change in synaptic strength between these two brain areas. My goal is now to manipulate synaptic plasticity at these afferences to unravel the synaptic mechanisms underlying memory consolidation in cortical associative areas.

A5 - Nanoscale organization of axo-axonic synapses along the AIS of cortical pyramidal neurons

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Synapses in the central nervous system are highly specialized structures dedicated to the transfer of information from one neuron to another. Each neuron receives thousands of excitatory and inhibitory synaptic inputs along their dendrites, that are integrated at the axon initial segment (AIS) to generate a new electrical signal called an Action Potential (AP). Regulation of this fundamental process is key for

proper brain function and was shown to be altered in several neurodevelopmental pathologies such as schizophrenia and autism spectrum disorder. Interestingly, the AIS of pyramidal neurons (PNs) is innervated by a specific type of inhibitory interneuron, a Chandelier cell. Although these axo-axonic synapses are thought to be capable of tightly regulating AP initiation, their structural and functional properties remain largely unexplored. At classical synapses formed along dendrites, the molecular organization of synaptic proteins at the nanoscale is a key factor in fine tuning the efficiency of synaptic transmission. However, the precise nanoscale arrangement of molecules at axo-axonic synapses has not been previously characterized, nor its impact on the modulation of pyramidal neuron firing. Here, we show that key proteins such as Gephyrin, the main scaffolding protein of inhibitory synapses, forms subsynaptic domains (SSD) at axo-axonic synapses. These SSDs display different properties, in term of size and density of proteins, when compared to dendritic synapses, which are formed by different types of interneurons. In addition, we show that Gephyrin SSDs can be reorganized in an activity-dependent manner in vivo. Our findings provide a potential mechanism by which Chandelier cells synapse tune their strength to control PN output.

A6 - Focus on the mouse insular cortex: *in vivo* single-unit recordings during anxiety assays and mapping of the dopaminergic system

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The insular cortex (insula) is over-activated in patients with anxiety disorders and preclinical observation showed that dysfunctions of dopamine (DA) transmission can alter anxiety level. DA receptors are widely expressed across the brain, but their role within the insula on anxiety remains unknown.

By performing extracellular recordings in freely-moving mice, we provide the first *in vivo* electrophysiological characterization of anterior insula neurons during anxiety assays. We found heterogeneity in single-unit firing profiles, depending on the location of the mice in safe or anxiogenic spaces, with a majority of units more active in anxiogenic spaces. Then, as an initial step to define the function of insula DA transmission in anxiety, we quantified dopamine 1 receptor expression in the insula using a transgenic D1:Ai14 mouse line. We found that **[1]** D1+ cells density is the highest in layer 2-3, **[2]** ~30% of inhibitory neurons are D1+, and **[3]** only 10% of excitatory neurons are D1+.

Finally, by combining tyrosine hydroxylase immunohistochemistry and retrograde tracing, we mapped the source of dopaminergic inputs to the insula, and by using anterograde viral tracing in D1-cre mice, we mapped the outputs of the 10% of D1+ projection neurons. We evidenced that, as for the whole projection neurons of the insula, D1+ insula projection neurons mainly target the amygdala and controlateral insula.

Together, this preliminary study provides an electrophysiological and anatomical characterization of insula neurons as a starting point to identify the potential role of dopaminergic modulation of the insula in anxiety.

A7 - Building stronger synapses: CaMKII & alpha-actinin-2 interactions underlying long-term potentiation

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Long-lasting changes in the strength of synaptic strength underlie learning and cognition. In excitatory synapses, synaptic plasticity is typically triggered be entry of Ca^{2+} through NMDA-type glutamate receptors (NMDARs). Large influxes of Ca^{2+} activate the highly abundant enzyme calcium/calmodulin-dependent kinase II (CaMKII), which drives long-term potentiation by phosphorylating key proteins to increase post-synaptic responsiveness. However, the kinase also plays a structural role – supporting the structure of enlarged synapses by virtue of its ability to nucleate multivalent interactions aided by its dodecameric structure (Hell, 2014). One important binding partner for CaMKIIα is the actin crosslinking protein α-actinin-2. Prior work has shown that α-actinin-2 associates with the CaMKIIα regulatory segment through its third and fourth EF hands, and that the interaction is competitive with Ca^{2+}/calmodulin (Jalan-Sakrikar et al., 2012). Since LTP is triggered by CaMKII activation by calmodulin, it is not therefore immediately apparent how and when interactions between CaMKII and α-actinin-2 contribute to stabilizing dendritic spine structure. In this study we focused on determining when CaMKII and α-actinin-2 associate, understanding the molecular basis of their interaction, and investigating the effects of disrupting interactions between the two. Proximity ligations assays in primary hipocsampal neurons showed that CaMKII and α-actinin-2 association increases markedly upon activation of NMDARs. We found that disruption of interaction between the two proteins prevented a transition from stubby to mushroom-type dendritic spines upon NMDAR activation, consistent with a key role for interaction between the two proteins in supporting the structure of potentiated synapses following long-term potentiation. We have also determined a high-resolution structure showing how α-actinin-2 binds to the CaMKII regulatory segment, revealing an unexpected binding mode. Taken together, our results highlight the importance of the structural role played by CaMKII in synaptic plasticity.

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Jalan-Sakrikar, N., Bartlett, R. K., Baucum, A. J., 2nd, & Colbran, R. J. (2012, May 4). Substrate-selective and calcium-independent activation of CaMKII by alpha-actinin. *J Biol Chem, 287*(19), 15275-15283. https://doi.org/10.1074/jbc.M112.351817

A8 - Glucose-derived glutamate drives synaptic local translation and neuronal maturation

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Neurons are extremely complex cells, whose organization and activity need to be finely tuned at metabolic level. The development, building and maintenance of their articulate architecture, as well as the rapid integration of exogenous and endogenous stimuli require a high energy demand. However, little is known about the molecular mechanisms involved in energy metabolism governing neuronal maturation. Here, we investigate the contribution of the main metabolic pathways, namely glucose, glutamine and fatty acids oxidation to the maturation of rat primary hippocampal neurons. Notably, we show that UK5099, an inhibitor of the mitochondrial pyruvate transporter (Mpc-1), reduces metabolic functions, which are associated with impairments in neuronal arborization, synapses formation and synaptic transmission. Moreover, we show that oxidative glucose metabolism in the mitochondrion is required for glutamate production that, in turn, ensures proper spine density, neuronal arborization and electrical activity, by promoting local protein synthesis. These results demonstrate the deep connection between neuronal maturation and energy metabolism, which is not only required to meet energy needs, but has a key role in the biosynthesis of metabolic intermediates ensuring the proper neuronal maturation and functioning.

A9 - Brain micro-anatomy revealed by 2-photon shadow imaging *in vivo*

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Getting an accurate, detailed and physiologically relevant view of brain structure and neuronal circuits is a major goal of modern neuroscience. Current large-scale connectomics efforts rely either on EM or MRI, which are either incompatible with live conditions or do not offer cellular resolution. Fluorescence microscopy allows for live imaging with cellular resolution *in vivo*, but has relied on positively labeling of a sparse set of cells, giving an incomplete and biased view of the anatomical organization of brain tissue.

Breaking this impasse, super-resolution shadow imaging (SUSHI) established a new paradigm to visualize tissue anatomy in brain slices with nanoscale resolution in an all-encompassing and panoramic way, based on fluorescence labeling of the ACSF and 3D-STED microscopy. Because of the stringent optical

demands of super-resolution microscopy, however, the approach has only been applied to living organotypic brain slices so far.

We have now extended the shadow imaging concept to the mouse brain *in vivo*, based on 2-photon shadow imaging (TUSHI) and labeling of the cerebrospinal fluid with a fluorescent membrane-impermeant dye. We present the optical details of the microscope, the labeling strategy for sufficiently bright and homogeneous inverted cellular contrast, as well as the cranial window technique and anesthesia formula for optically clear and mechanically stable access to superficial layers of the cerebral cortex. Despite the diffraction-limited resolution, the new approach opens a stunning window on the micro-anatomical organization of the brain *in vivo*, where cell bodies, dendritic branches of neurons, perivascular spaces and spatial heterogeneities in the extracellular space become visible. By adding a second fluorescence channel, the shadow imaging approach reveals the diverse and complex anatomical context of positively labeled neurons, astrocytes, microglia and tumor cells.

In summary, our work demonstrates the feasibility of TUSHI in vivo to visualize brain structure and context with subcellular resolution. It provides a powerful new investigative tool to monitor dynamical changes of brain structures in vivo under various (patho-physiological) conditions, such as experience-dependent neuronal plasticity, sleep, aging, stroke, tumor invasion & proliferation.

A10 - Dynamics and nanoscale organization of neuroligin-1 adhesion molecules at synapses using high-end fluorescence imaging and computer simulations

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Neuroligins (NLGNs) form a family of cell adhesion molecules implicated in synapse development, but the mechanisms that retain these proteins at synapses are still incompletely understood. Recent studies indicate that surface-associated NLGN1 is diffusionally trapped at synapses, where it interacts with quasi-static scaffolding elements of the post-synaptic density. Whereas single molecule tracking reveals rapid diffusion and transient immobilization of NLGN1 at synapses within seconds, fluorescence recovery after photobleaching experiments indicate instead a long-term turnover of NLGN1 at synapse, in the hour time range. To gain insight into the mechanisms supporting NLGN1 anchorage at post-synapses and try to reconcile those experimental paradigms, we quantitatively analyzed here live-cell and super-resolution imaging experiments performed on NLGN1 using a newly released simulator of membrane protein dynamics for fluorescence microscopy, FluoSim (Lagardère et al. Sci Rep 2020). Based on a small set of parameters including diffusion coefficients, binding constants, and photophysical rates, the framework describes fairly well the dynamic behavior of extra-synaptic and synaptic NLGN1 over both short and long-time ranges, and provides an estimate of NLGN1 copy numbers in post-synaptic densities at steady-state (around 50 dimers). One striking result is that the residence time of NLGN1 at synapses is much longer than what can be expected from extracellular interactions with pre-synaptic neurexins only. Furthermore, a GPI-anchored NLGN1 molecule shows increased surface diffusion and decreased synaptic enrichment. Together, these results indicate that NLGN1 is stabilized at synapses through multivalent interactions with intracellular post-synaptic scaffolding proteins

(Lagardère/Drouet et al., Frontiers Synaptic Neurosci 2022). Whereas our initial imaging strategy relied on the replacement of endogenous NLGN1 using shRNA plus rescue with fluorescently tagged NLGN1 molecules in hippocampal neurons, we are now using cultures from a newly generated knock-in mouse in which native NLGN1 has been fused to a small N-terminal biotin acceptor peptide (BAP), allowing its targeted biotinylation and subsequent labeling with streptavidin conjugates.

References

Lagardère M., Chamma I., Bouilhol E., Nikolski M., Thoumine O. (2020). **Sci. Rep.** 10:19954

FluoSim: simulator of single molecule dynamics for fluorescence live-cell and super-resolution imaging of membrane proteins.

Lagardère M., Drouet A., Sainlos M., Thoumine O. (2022). **Front. Synaptic Neurosci.** 10.3389

High-Resolution Fluorescence Imaging Combined With Computer Simulations to Quantitate Surface Dynamics and Nanoscale Organization of Neuroligin-1 at Synapses.

A11 - A new mouse line to re-explore the localization and function of endogenous Neuroligin-1

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Synapses are established and maintained by adhesion molecules called synaptic organizers, which include pre-synaptic neurexins and post-synaptic neuroligins (NLGNs). In the absence of highly specific antibodies to these molecules, the sub-cellular distribution and synaptic function of NLGNs have been extensively manipulated using knock-down, knock-out, over-expression or rescues approaches, leading to controversial results. In this context, we generated a new transgenic knock-in mouse strain in which endogenous NLGN-1 has been N-terminally tagged with a small biotin acceptor peptide (BAP) by gene editing using the CRISPR-Cas-9 strategy. In this system, native NLGN1 can be enzymatically biotinylated by the viral delivery of biotin ligase, allowing for its subsequent high affinity labeling by streptavidin conjugates. By taking advantage of complementary assays including behavioral assessment, biochemistry, immunohistochemistry, confocal microscopy and transmission electron microscopy, we performed an exhaustive characterization of this new mouse line focusing on characteristic regions where NLGN-1 is highly expressed (i.e. hippocampus, cerebellum and cortex), and found that addition of the BAP tag does not seem to affect brain and synapses development. Then, using primary hippocampal neurons cultured from BAP-NLGN-1 knock-in mice infected with a virus encoding the biotin ligase ($BirA^{ER}$-IRES-GFP), we show an extremely efficient pull-down of biotinylated BAP-NLGN1 by streptavidin beads in biochemistry, and a selective surface labeling of biotinylated BAP-NLGN-1 with fluorescent streptavidin. Interestingly, by using a co-labelling with PSD-95 or gephyrin, our preliminary results suggest that NLGN-1 is targeted to both excitatory and inhibitory synapses. We will further use this new transgenic knock-in mouse line to revisit the role of NLGN-1 in synapse differentiation that we

previously addressed using knock-down and rescue approaches (*Letellier et al., Nat Commun 2018; Toledo et al., eLife 2022*).

A12 - New methods for super-resolution imaging of AMPA receptors in synapses: Expansion Microscopy and Electron microscopy tomography.

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Conventional super-resolution methods, such as Single Molecule Localization (SML) and Stimulated Emission Depletion microscopy have revolutionized the field of biological imaging. We have observed molecules at nanoscale level thanks to the development of new fluorescence probes, new microscopy approaches and new sample preparation methods. However, in addition of the need of high technical expertise and expensive equipment, these techniques present some limitations in 3D imaging and the lack of the cellular context. At Bordeaux Imaging Center, we are working on these limitations and looking for smart alternatives to these technical approaches.

Expansion microscopy is a simple and cheap alternative to fluorescence superresolution. Developed by Ed Boyden in 2014 for the first time, there are currently many different approaches depending on the level of resolution required and the biological sample. This method is based in mechanical expansion of the biological material. This way, we can use conventional microscopy such as epifluorescence and confocal systems to observe the samples at 4-20 folds their size.

On the other side, the highest resolution in imaging is obtained by electron microscopy. However, there are some limitations in obtaining the specific information of the molecule of interest. We have developed at the BIC, pre-embedding labeling of AMPA receptors to be observed by Scanning Transmission Electron Microscopy and Tomography (STEM tomography).

By using these methods at the BIC, we are able to obtain super-resolved images of AMPA receptors in their ultrastructural context.

A13 - NMDA receptors nanoarchitecture: an emerging regulator of glutamatergic synapses

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The ionotropic NMDA receptors (NMDAR) greatly contribute to the excitatory glutamatergic drive in the central nervous system. Their function is crucial for neuronal physiology and synaptic plasticity. In addition, their dysfunctions have been associated with the etiology of several major neurological and psychiatric diseases. Therefore, understanding how these receptors are regulated in healthy and diseased brains has been a major challenge for many laboratories over the past decades.

Recent developments in the super-resolution microscopy field have revealed that beyond the diffraction-limited view, NMDARs are organized into nanometer-sized clusters, termed nanodomains (Kellermayer*, Ferreira*, Dupuis* et al., 2018). We used super-resolution imaging and single nanoparticle tracking in rat hippocampal neurons to unveil the nanoscale topography of native GluN2A- and GluN2B-NMDAR along the dendritic arbor. We have explored how distinct nanoscale organizations are associated with modifications to synaptic plasticity, namely long-term potentiation (LTP). Our data reveal that the nanoscale organization of NMDARs changes alongside the dendritic segments in a subtype-specific manner and is shaped by the interplay with CaMKII at proximal dendritic segments, shedding new light on our understanding of the functional diversity of hippocampal glutamatergic synapses (Ferreira et al., 2020).

Our recent studies reveal that the dynamic nature of GluN2B-containing NMDARs (GluN2B-NMDAR) makes them key players for the NMDAR-dependent regulation of synaptic adaptation. We have shown GluN2B-NMDARs are important molecular hubs for the synaptic localization of the proteasome (Ferreira et al., 2021). The various interplays of NMDAR with intracellular proteins are instrumental for the receptor function. Understanding how NMDAR nanoarchitecture influences synaptic signaling in neurons will shed new lights on how to regulate and modulate glutamatergic synapses composition.

A14 - MeCP2 deficiency in astrocytes alters synaptogenesis: new insights on Rett syndrome

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Rett syndrome (RTT; OMIM# 312750) is a rare devastating neurodevelopmental disorder that represents the most common genetic cause of severe intellectual disability in girls. Mutations in the X-linked methyl-CpG-binding protein 2 (*MECP2*) gene have been reported in over 95% cases of classical forms of RTT. Although initial studies supported a role for MeCP2 exclusively in neurons, recent data indicate a function also in astrocytes, which emerged as critical players involved in RTT pathogenesis through non-cell autonomous effects. Nevertheless, many aspects of RTT astrocyte dysfunctions remain unknown. RTT is considered a synaptopathy characterized by dendritic spine dysgenesis, impaired spine plasticity, alteration of neuronal excitability and disrupted excitatory/inhibitory balance of circuits. According to the crucial role of astrocytes in promoting synapse formation and functioning, we have investigated the influence of *Mecp2* null astrocytes on synaptic phenotype. By using *in vitro* transwell-based co-cultures or neuronal treatment with astrocyte conditioned medium (ACM), we have demonstrated that the lack of *Mecp2* in cortical astrocytes dramatically influences the synaptogenesis of WT neurons. In particular, KO astrocytes release one or more thermo-labile factors, that detrimentally affect both the pre- and post-synaptic terminals. To gain insights on the involved molecular mechanisms, we used an indirect approach, by performing bulk RNA-Sequencing on WT neurons co-cultured with KO astrocytes. We have thus profiled the molecular pathways activated in WT neurons by the paracrine effects triggered by KO cortical astrocytes and by bioinformatic analyses we confirmed that KO astrocytes influence neuronal pathways mainly associated with synaptic maturation and functions and elicit inflammatory responses. qPCR and luminex assay indicated the up-regulated release of a subset of cytokines by null astrocytes, with a possible synaptotoxic effect. We are currently validating the role of two of these proteins on the occurrence of synaptic defects, being aware that the identification of the involved factors might reveal novel therapeutic approaches for the treatment of RTT.

A15 - Rabphilin 3A: Linking NMDA Receptor Synaptic Retention to Synaptic Plasticity and Brain Diseases

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Synaptic transmission and plasticity at glutamatergic synapses are strictly correlated with the correct synaptic localization of GluN2A-containing NMDARs. Accordingly, aberrant synaptic trafficking and stabilization of the GluN2A subunit have been associated with both neurodevelopmental and neurodegenerative disorders. Our group recently identified Rabphilin3A (Rph3A) as a novel GluN2A synaptic partner that stabilizes synaptic NMDARs at cell surface through the formation of a ternary complex with GluN2A and PSD95 (Stanic et al., 2015; Franchini et al., 2019, 2022). Formation of Rph3A/NMDAR complex is needed for the induction of long-term potentiation (LTP) and NMDAR-dependent hippocampal behaviors, such as spatial learning. Modulation of Rph3A expression leads also to bidirectional alterations of dendritic spine density. In particular, Rph3A overexpression in primary hippocampal neuronal cultures is sufficient to induce molecular modifications at NMDARs leading to dendritic spine formation and the occlusion of LTP-induced modifications of spine density.

Moreover, we recently identified a specific role for Rph3A in both neurodevelopmental and neurodegenerative diseases. Through exome sequencing, we identified two *de novo* variants (T450S, N618S) in *RPH3A* in individuals affected by neurodevelopmental disorders with untreatable epileptic seizures. Interestingly, both mutations lead to aberrant localization of GluN2A-containing NMDARs at extrasynaptic membranes (Pavinato et al., submitted). Finally, we identified Rph3A as a novel mediator of α-synuclein-induced NMDAR-endocytosis leading to postsynaptic toxicity both in the striatum and the hippocampus. Rph3A overexpression and the use of a small molecule able to interfere with the Rph3A/αsyn complex are sufficient to fully prevent dendritic spine loss and associated early motor defects in an animal model of synucleinopathy (Ferrari et al., submitted). Overall, our results identify Rph3A as a novel postsynaptic protein linking NMDAR synaptic retention to synaptic plasticity and brain diseases.

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A16 - Cerebellin-4 in the prefrontal cortex maintains excitatory and inhibitory synaptic transmission critical for memory-guided behaviours

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Neuropsychiatric disorders are marked by a combination of behavioural symptoms. One such behavioural abnormality that is common to a continuum of neuropsychiatric disorder is an impairment in learning and memory. However, an understanding of the synaptic and circuit pathophysiology underlying these abnormalities is limited. Many of these disorders also share common genetic defects, some of which result from dysfunctional synaptic cell adhesion molecules (CAMs) such as neurexins (Nrxns) and their interacting partners. One family of neurexin-interacting proteins that are implicated in a multitude of neuropsychiatric disorders are cerebellins (Cbln1, Cbln2, Cbln3 and Cbln4). Cerebellins are glycoproteins containing a C1q domain and are thought to be secreted presynaptically. These proteins have been implicated in synapse formation, maintenance and activity-dependent plasticity. Despite observed penetrance in disease, the synaptic mechanisms by which Cbln isoforms mediate healthy behaviour is poorly understood. Specifically, Cbln4 presents as an enigma owing to its expression in regions implicated in learning and memory – including the prefrontal cortex and the basolateral amygdala, as well as a lack of understanding of its function and interactions at synapses.

To study the putative role of Cbln4 in the medial prefrontal cortical (mPFC) ensembles mediating learning and memory, we utilized a conditional knockout mouse for Cbln4 (Cbln4-cKO). In these mice, we deleted Cbln4 from the mPFC by stereotaxic injection of adeno-associated viruses (AAV) encoding Cre-recombinases. We show that Cbln4 plays a crucial role in defining synaptic properties at both the excitatory and the inhibitory synapses in the medial prefrontal cortex (mPFC). Specifically, when Cbln4 was deleted from the mPFC, we saw a decrease in the frequencies of miniature EPSCs and miniature IPSCs, without any significant changes to their amplitudes. In mice with mPFC Cbln4 deletion, we saw an impairment in cue-associated fear learning, whereas contextual fear learning and contextual fear extinction were unperturbed, implicating a role for mPFC Cbln4 in cue-associated learning and memory.

These results demonstrate that Cbln4 plays an important role in maintaining synaptic transmission at both excitatory and inhibitory synapses in the mPFC. Moreover, behavioural deficits observed as a consequence of Cbln4 deletion in the mPFC are likely to play an important role in the pathophysiology underlying the complex neuropsychiatric disorders caused by Cbln4 mutations.

A17 - Unraveling AMPA receptor dynamics in striatal neurons during Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder that clinically manifests with motor impairments. Functional imaging studies in human PD patients revealed altered connectivity between the cortex and striatum. In mice, expressing a knock-in mutation, *Lrrk2*^{G2019S}, which in humans is one of the most common genetic determinants of PD, we found that it disrupts long-term potentiation (LTP) of cortical synapses onto striatal spiny projection neurons (SPNs), a deficit that may contribute mechanistically to cognitive and psychiatric non-motor symptoms of PD in humans. We found that at baseline, glutamatergic synapses on *Lrrk2*^{G2019S} D_1R-SPNs show an abnormally high accumulation of surface GluA1, while total GluA protein levels remain unchanged. This effect is robust as it is detected in intact slices and in cultured neurons, using electrophysiological, pharmacological, biochemical, and cell biological approaches. Mechanistically, this increase is driven in part by a failure to internalize and recycle GluA1 subunits. It is also selective, as no changes are observed in surface GluA2, and transferrin receptors recycle normally. Thus, disruption in AMPAR stoichiometry and trafficking at baseline and in response to long-term plasticity, could mechanistically contribute to the non-motor behaviors of PD.

A18 - Neurotransmitter release in synapse formation of emerging neuronal circuits

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The establishment of synapses between two different neurons is crucial for the correct wiring of neuronal circuits and communication throughout the brain. It has long been evident that neuronal activity plays a critical role in the refinement and maintenance of synaptic connections, but more recently it is becoming clear that activity may also be important for synapse and circuit formation. Neuronal activity can be separated into different forms; action potential dependent neurotransmitter release (synchronous) and spontaneous fusion of vesicles. These two modes of vesicle cycling are derived from distinct pools with different levels throughout development, dramatically changing from high-levels of spontaneous cycling in young neurons to the mature-phenotype with strong evoked-

release. By conditionally eliminating either fast, synchronous evoked release, synaptotagmin 1 deletion, or abolishing all release, Munc18-1 deletion, we have examined, *in-vivo*, the impact of different aspects of neuronal activity on dendritic development and synapse formation within a specific circuit of the developing hippocampus (Prox1-Cre driver lines). We reveal that manipulation of the different modes of neuronal activity within a critical time window alters synapse formation, indicating an important role for the modes of glutamate release for synaptic connections.

A19 - Cyclin G2 May Influence Synaptic Density Via Its Interaction with β-Catenin

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Cyclin G2 (CycG2) is an unconventional cyclin homolog well expressed in brain. In non-neuronal cells. CycG2 gene (*CCNG2*) expression is upregulated during cellular stress and growth inhibitory responses and promotes G_2-phase and G_1-phase cell cycle arrest responses to DNA damage and pharmacological blockade of proliferation pathways. We found that in rodents CycG2 gene (*Ccng2*) transcripts are increased during cerebellar development, reaching peak levels as granule cell precursors exit the cell cycle and differentiate into neurons. We determined by immunoblot analysis and immunofluorescence microscopy that CycG2 protein expression is abundant in hippocampal and cerebellar tissues and neurons. As our previous work demonstrated that CycG2 forms a catalytically active complex with the serine/threonine phosphatase PP2A/B' and C subunits in cerebellar tissues, we examined whether neuronal CycG2 interacts with other PP2A associated proteins, including β-catenin. Intriguingly, parallel studies of others in non-neuronal mitotic cell types linked CycG2 with β-catenin/Wnt signaling functions, repressing β-catenin nuclear functions while augmenting its role in non-neuronal cell adhesion. Through immunoprecipitation, pulldown experiments and fluorescence polarization assays we found compelling evidence that CycG2 directly associates with β-catenin, and that β-catenin binds just N-terminal to the PP2A/C and B' subunit binding sites in the carboxy terminus of CycG2. We have narrowed down the binding regions for beta-catenin and PP2A subunits, including the SLIM binding motif for the B' subunit of PP2A immediately upstream of the carboxyterminal PEST domain. Preliminary evidence obtained from imaging of Golgi-stained brain tissues of *Ccng2* KO mice indicates that KO neurons exhibit decreased spine density compared to that in WT animals. Ongoing work includes further assessment of neuronal morphology and spine density of hippocampal neurons from additional age-matched Ccng2 WT and KO mice and how CycG2 loss may affect the localization and interactions of its neuronal binding partners.

A20 - Molecular mechanisms of drug-induced synaptogenesis

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Relapse is a major challenge to the treatment of substance use disorder, mainly triggered by drug-associated memories, which contrary to physiological memories, exhibit an exceedingly rigid and long-lasting nature as a product of maladaptive plasticity. Repetitive cocaine exposure generates *de novo* AMPA receptor-silent synapses in the nucleus accumbens[1], whose maturation underlies drug-associated behaviors[2]. As silent synapses provide novel synaptic opportunities, we hypothesize that drug-induced synaptogenesis reorganizes neural networks to change the connection patterns morphologically and functionally, and as a result encode drug-associated memories with rigid and long-lasting features. Using the electrophysiology procedure of minimal stimulation[3], we describe repetitive cocaine exposure has more generalized implications than previously reported, as we found that silent synapses are generated in additional brain areas, including the dorsal striatum and the dorsal hippocampus. Obtaining a blueprint of the molecular mechanisms underlying silent synapse generation will help to unveil the consequences of silent synapse-based network reorganization in substance use disorders. We report that viral vector-mediated knockdown of the gabapentin receptor α2δ-1 prevented the synaptogenic effect of cocaine in the dorsal striatum. These results suggest a glial-neuronal crosstalk to be involved in the signaling pathway for the generation of silent synapses.

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A21 - Presynaptic plasticity in the pathophysiology of the Fragile X Syndrome

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Fragile X Syndrome (FXS) is the most frequent form of inherited intellectual disability (ID) and a leading cause of autism spectrum disorder (ASD). FXS is caused by the silencing of the Fragile X Mental

Retardation gene (FMR1) which encodes the RNA-binding protein FMRP. Fmr1-KO mice recapitulate the disorder and are a model of both ID and ASD (Dahlhaus, 2018). The project, which is part of a national collaboration, aims at characterizing the functional implication of novel targets of FMRP. Here we focus on targets which could play a role in presynaptic forms of synaptic plasticity, with a primary focus on a recently identified target, Phosphodiesterase 2A (PDE2A) (Maurin et al., 2018). Most studies in the context of FXS have related to dendritic and post synaptic mechanisms. However, PDE2A is located in docked synaptic vesicles (Boyken et al., 2013) and highly expressed in brain regions involved in FXS pathology (Stephenson et al., 2012). The role of PDE2A in functional and structural presynaptic mechanisms needs to be further explored in Fmr1-cKO. The specific aim of the project is to unravel the impact of dysregulated PDE2A induced by the absence of FMRP on structural and functional presynaptic mechanisms and plasticity in the CA3 region of the hippocampus. Here we will test whether presynaptic mechanisms and plasticity are altered in Fmr1-cKO mice. In addition, correction of phenotypes (if any) driven by the absence of FMRP, will be carried out by reducing the expression level or activity of PDE2A. The project will use a combination of patch-clamp electrophysiology and 2-photon imaging in slices with behaviour, in mice with cell-specific gene mutations targeted to the dentate gyrus and CA3.

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A22 - Expanding the protein-based fluorescent probe toolkit for advanced synapse imaging

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Fluorescence microscopy arguably constitutes a cornerstone technique of modern neurobiology. With the recent instrumental and technological progress that now provide access to advanced imaging modalities, there is a pressing need for a novel generation of probes that would allow to fully benefit from the resulting gains in resolution and multiplexing capacities.

In this context, the development of novel protein-based fluorescent probes to label synaptic proteins for super-resolutive microscopy applications has been a major focus of our research efforts. In particular, by addressing some of the main limitations of current probes, we aim at providing a complementary set of biomolecular tools to investigate proteins of interest either genetically modified or in their endogenous

form. To achieve our goals, we combine rational probe design together with directed protein evolution to generate binders of small size generic epitope tags or directly epitope from a protein of interest. Following in-depth characterization and validation of the new binders, we next further engineer them to produce ready to use fluorescent probes for imaging applications. We present here an overview of the tools and strategies that we have developed so far together with our recent progress in improving and expanding the protein labeling toolkit.

A23 - The Neurotrophic Molecule sAPPα Regulates the Expression of AMPA Receptors to Enhance Plasticity

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Regulation of AMPA receptor expression by neuronal activity and neuromodulators is critical to the expression of both long-term potentiation (LTP) and memory. In particular, Ca^{2+}-permeable AMPA receptors (CP-AMPAR) play a unique role in these processes due to their transient, activity-regulated expression at synapses[1]. Secreted amyloid precursor protein-alpha (sAPPα), a metabolite of the parent amyloid precursor protein (APP) has been previously shown to enhance hippocampal LTP, and rescue impaired memory, as well as promote trafficking of GluA1-containing AMPARs to the cell surface and specifically enhance the synthesis of GluA1[2].

In recent work, using fluorescent non-canonical amino acid tagging–proximity ligation assay (FUNCAT-PLA), we show that brief treatment of primary rat hippocampal neurons with sAPPα (1 nM, 30 min) rapidly enhances the dendritic cell-surface expression of *de novo* GluA1 homomers (3.97 ± 0.32, p = 0.0009), and reduces levels of *de novo* GluA2 (0.54 ± 0.17, p = 0.009), as well as extant GluA2/3-AMPARs (0.63 ± 0.12, p = 0.0033), while longer exposure to sAPPα increased levels of GluA1/2 AMPARs (120 min; 2.28 ± 0.14, $p \leq 0.0001$). Moreover, sAPPα-mediated enhancement of the induction of LTP in area CA1 of acute hippocampal slices was dependent on the activation of these CP-AMPARs[3].

To further understand how sAPPα regulates AMPAR expression, we aimed to examine the characteristics of AMPAR clusters at the neuronal cell surface utilizing super-resolution (dSTORM) microscopy. Here, we found an upregulation in the number of GluA1 AMPAR clusters following sAPPα treatment (30 min; 1.04 AMPAR/μM^2 ± 0.07, p = 0.0038), which was further enhanced when followed by a chemical LTP protocol (cLTP; 1.63 ± 0.18, p = 0.0028), likely indicating that sAPPα drives both *de novo,* as well as stored, AMPAR to the cell surface following synaptic activity. Interestingly, no changes were detected in the number of AMPAR per cluster, or the packing density. This work expands upon our current knowledge of how sAPPα regulates the expression of plasticity through the coordinated synthesis and expression of GluA1 clusters at the cell surface.

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A24 - Developmental expression of SV2 isoforms in iPSC-derived human neurons

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Epileptic seizures are important symptoms of several major brain disorders, where synaptic dysfunction becomes a central factor leading to dysfunction of neuronal networks[1]. The synaptic vesicle (SV) protein 2 family, a class of glycoproteins that are unique for vertebrates[2], are strongly linked with epilepsy but their function remains largely unknown. Three major isoforms are expressed in the human brain: SV2A (present in glutamatergic and GABAergic neurons), SV2B (glutamatergic neurons), and SV2C (GABAergic, cholinergic, and dopaminergic neurons)[2]. In mice, the absence of SV2A leads to recurring epileptic seizures after birth and eventually death[3]. In humans, mutations in the SV2A gene lead to epilepsy[4,5,6] and SV2A is the target of a class of anti-epileptic drugs (AEDs)[2]. Work in rodents suggests that SV2A may have a role in facilitating synaptic release[7,8], however there is a major lack of information regarding SV2A function in human neurons. Here, we examine the role of SV2A in induced pluripotent stem cell (iPSC)-derived human neurons, starting by characterizing the expression profile of the three SV2 isoforms throughout development. Neurons were generated by forced expression of NGN2 in BIONi010-C-13 iPSCs and harvested at different time points post-induction to evaluate mRNA and protein levels. Additionally, homozygous and heterozygous knockout (KO) models for SV2A were generated in the same iPSC line using CRISPR/Cas9. We are currently in the process of analyzing SV2A expression and function in these models with whole-cell patch-clamp and live cell imaging. With this work we aim to better understand the role of SV2A in synaptic transmission during development in human neurons.

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A25 - New insights into the role of the proprotein convertase subtilisin/kexin type 9 (PCSK9) in neurons

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The brain is the most cholesterol-rich organ and, at the synapse level, cholesterol is a component of lipid rafts, that are important for synaptic function and spine morphology. Adult neurons are dependent on cholesterol obtained from astrocytes carried through ApoE particles incorporated into neurons through definite receptors. In the brain ApoE binds numerous low-density lipoprotein receptors (LDLRs), such as LDLR, LRP1, ApoER2. These receptors play a variety of roles outside of lipid trafficking and metabolism, including synaptic transmission and modulation of spine structure.

LDLRs function can be regulated by the Proprotein convertase subtilisin/kexin type9 (PCSK9). PCSK9 enhances the degradation of LDLRs and, thereby, reduces the clearance of low-density lipoproteins. However, even though the brain expresses high levels of PCSK9, the role of this protein in neurons has not clearly described yet.

To investigate the impact of PCSK9 on neuronal function, we transfected primary hippocampal cultures with PCSK9 shRNA. In neurons, PCSK9 downregulation leads to an altered synaptic localization of glutamate receptor's subunits. Furthermore, the silencing of PCSK9 in hippocampal neuronal cultures decreases spine density and changes the morphology of dendritic spines.

In addition, we generated a new transgenic line lacking PCSK9 selectively in neuronal cells (CaMKIICre+/Pcsk9$^{LoxP/LoxP}$ mice). These mice have reduced PCSK9 expression in hippocampus and cortex, but circulating levels are not affected. The lack of PSCK9 impairs spatial memory, as shown by object location task, affects spine morphology, NMDA receptors synaptic localization and synaptic plasticity pathways in the hippocampus. Remarkably, ApoER2 is the only target of PSCK9 showing an increased localization in the synaptic compartment. Experiments performed in primary hippocampal cultures revealed that PCSK9 downregulation affects ApoER2 synaptic membrane expression and lipid droplets levels.

These results disclose the relevance of PCSK9 in the brain and suggest that PCSK9 can be novel key regulator of synaptic structure and function.

A26 - Context-dependent neuronal actvity of ventral hippocampus underlying reward and aversive Memories

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Memory-guided behavior depends on the brain's capability to simultaneously construct salient emotional experiences and internal representations of encountered spatial environments. The ventral hippocampus (vHip) plays an important role in emotional behaviors associated with rewarding and aversive experiences. However, the neuronal dynamics and circuits within the vHip underlying the formation of context-dependent emotional memories remain poorly understood.

Using in vivo single-unit recordings in freely behaving mice to monitor neuronal activity during a contextual reward- and fear conditioning paradigm, we found that neuronal activity in vHip discriminates between emotional contexts with distinct valence. Contextual neuronal activity in vHip did

preferentially form in emotion-contingent contexts after learning and a support-vector-machine based classifier was able to predict context identity.

We next identified which pre-synaptic input to vHip mediate the formation of emotional contextual memories. To do so, we optogenetically manipulated the locus coeruleus (LC), a brain region known to release noradrenalin and regulate hippocampal plasticity and learning. We observed that suppression of LC activity in vHip during learning impaired contextual reward memory formation, although contextual fear memory was preserved. These findings identify selective neuronal activity patterns in vHip underlying emotional contextual memories and provide evidence that synaptic inputs from the LC to vHip plays a key role in contextual reward memory

A27 - The wnk pathway tunes $gaba_a$ receptor diffusion and nanoscale organization at hippocampal gabaergic synapses

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It has been established that the chloride-sensitive WNK/SPAK pathway rapidly tunes the neuronal Cl- homeostasis by regulating the chloride cotransporters KCC2 near the GABAergic synapse, thereby affecting the efficacy of the GABAergic synapses (Heubl et al., Nature Comm 2017, 8: 1776). However, it is still unknown whether this pathway controls the GABAergic synapse by targeting GABAAR or the gephyrin scaffold.

Immunocytochemistry labelling of gephyrin clusters after pharmacological inhibition of the WNK1 and/or SPAK kinases in primary cultures of hippocampal neurons showed a modest decrease of clustering. Stochastical optical reconstruction microscopy (STORM) revealed that blocking the pathway leads to a reorganization of GABAAR clusters. Although the lateral diffusion of the GABAAR, assessed by Single-Particle Tracking (SPT) on live neurons, showed no effect at GABAergic synapses upon blockade of the pathway, extrasynaptic receptors had a decreased lateral diffusion. These results suggest a trapping of the receptor in endocytic wells, forming reserve pools at the membrane. This hypothesis has been confirmed by SPT experiments, using a peptide blocking clathrin-dependent endocytosis. Overall, these experiments suggest that the WNK pathway stabilizes GABAAR at the membrane.

To confirm these results, we activated the pathway using an extracellular solution depleted in Cl-. Our results showed that, contrary to the results of the WNK/SPAK activity blockade, activating the pathway increased the synaptic confinement of GABAARs. The effects of low chloride condition on extrasynaptic GABAAR are opposite to the ones found upon a blockade of the pathway.

The impact of the activation of the WNK/SPAK pathway on GABAAR clustering shows an increased clustering of GABAAR in super-resolution microscopy, confirming our hypothesis. However, the underlying molecular mechanisms of this regulation at the GABAergic synapse still remain unknown. We are currently investigating gephyrin putative phosphorylation sites to answer this question.

A28 - Towards understanding ribosome heterogeneity and specialization in dendrites

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Plasticity-induced differential recruitment of local protein synthesis-dependent plasticity is thought to drive synapse-specific phenotypes; however, the cellular mechanisms that underlie this supposed specificity are largely unknown. A promising candidate mechanism for sculpting synapse-specific proteomes is the selective translation of synaptic mRNAs by "specialized" ribosomes. Ribosomal protein (RP)-encoding mRNAs are locally translated in rat hippocampal neurons and can be incorporated into existing, actively-translating ribosomes in a context-dependent manner (Fusco et al., 2021). Furthermore, a subpopulation of neuronal ribosomes (monosomes) is now known to preferentially translate neuropil-localized transcripts important in plasticity (Biever et al., 2020; Glock et al., 2021); however, whether and how they are compositionally distinct from other ribosomes (namely polysomes) remains unclear.

Here, we set out to uncover heterogeneity in ribosome composition through two biochemical and proteomic approaches. In the first approach, we assess the distribution of ribosome-associated proteins across multiple, functionally distinct ribosomes subcomplexes, including the small and large subunits (40S and 60S), monosomes (80S), and polysomes. We are analyzing cytoplasmic lysates from cultured rat cortical neurons by polysome proteome profiling (3P; Imami et al., 2018), which couples sucrose gradient fractionation with SILAC-based quantitative proteomics, to obtain a high-resolution map of the neuronal ribo-interactome. In the second approach, we combine proximity labeling catalyzed by synapse-targeted APEX2, ribosome purification, and mass spectrometry to identify the RPs and binding proteins associated with ribosomes found at or near the synapse. Future studies will combine ribosome profiling, immunopurification, and proteomics to test the hypothesis that plasticity modifies the synaptic ribosome-proteome to 'tune' the local translatome.

A29 - Imaging local protein synthesis in the mouse cortex

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Synaptic plasticity is a key process for brain function and memory formation. This, in turn, depends on the ability of neurons to remodel their synaptic proteomes with high temporal and spatial resolution, promptly delivering and degrading the right proteins at the correct locations. Decades of work have shown that local protein synthesis at synapses is one of the mechanisms that neurons use to address this need. *In vitro* and *ex vivo* studies have shown that neurons locally translate thousands of mRNAs,

and that this process is required for several forms of long-term synaptic plasticity. In order to understand the role of local protein synthesis in physiological brain function and learning, it is required to study this phenomenon *in vivo*, in an intact neuronal circuit. Here, we present our efforts to visualize directly local protein synthesis dynamics of mRNAs in awake mice. Using protein synthesis reporters fusing the mRNA encoding a fast-folding fluorescent protein to the 3'untranslated regions of two synaptic transcripts, *Beta actin* and *Psd95*, we localize this reporter mRNA into distal neuronal compartments. By sparsely expressing these reporters in deep layer excitatory neurons of the auditory cortex, and then imaging > 400um away from the neuronal somata we can achieve sufficient spatiotemporal resolution to assess the effect of local protein synthesis of our reporters. Using 2-photon imaging to perform fluorescent recovery after photobleaching (FRAP) in layer 1, we detect the robust local synthesis output of our reporters in these most apical dendrites. With this tool, for the first time, we can directly study the functional role of local protein synthesis in real-time *in vivo*.

A30 - The interplay between protein synthesis and proteasomal degradation in neuronal proteostasis

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Most catalysed chemical reactions inside cells depend on protein levels, and fine-tuning protein concentrations is key to ensure proper cellular function. The dynamic regulation of a balanced and functional proteome (i.e. proteostasis) concerns all proteins whose levels need to be adjusted in space and time in response to intracellular and extracellular cues. Precise control of these parameters is already challenging in cells that have little or no polarity, but becomes a particularly impressive feat for highly polarized cells, such as neurons.

To understand how the protein synthesis and the ubiquitin-proteasome systems (UPS) interact to effect neuronal proteostasis, we are asking how the protein synthesis machinery responds to the selective destabilization of a single protein substrate important for synaptic function. To this end, we are making use of two alternative proteolysis targeting technologies to destabilize endogenous CaMKIIα, Dlg4 and Gephyrin. First, we combined the recently developed dTAG system with the photoconvertible fluorescent protein Dendra2 to visualize drug-inducible degradation of CaMKIIα in rat primary neurons. In parallel, we optimized a protocol that allows for the differentiation of mouse embryonic stem cells into complex cultures harboring glia and mature inhibitory and excitatory neurons. We are now engineering the dTAG system at the genomic CaMKIIα locus of mESCs to achieve on-demand destabilization of endogenous CaMKIIα protein and study the protein synthesis response. In a second, complementary approach we are making use of the E3 ubiquitin ligase XIAP fused to antibody-like proteins (FingRs) to reduce the half-life of the synaptic scaffold proteins Gephyrin and Dlg4. Once these tools have been fully established and validated, we will perform puromycylation-PLA (Puro-PLA) to monitor the translation of the chosen synaptic targets following destabilization.

By combining different destabilization approaches to target several synaptic proteins we hope to better understand how the protein synthesis machinery and the UPS act coordinately to ensure neuronal proteostasis.

A31 - Transcriptomic characterization of synapse types and states

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Neurons are remarkably complex cells. Their intricate morphology and the demands of synaptic plasticity pose unique challenges for synaptic proteostasis and proteome remodeling. A critical mechanism in neurons to meet these demands is mRNA transport from the soma to neuronal processes and subsequent local protein synthesis near or at synapses. Recent technical advances have pushed the transcriptomic characterization of neuronal subcellular compartments with neurotransmitter type specificity and under different plasticity contexts. However, a comprehensive characterization of synapse type- and state-specific transcriptomes is still lacking. To address this, we use mouse lines with fluorescently tagged presynaptic terminals in a cell-type specific manner. We then dissect the brain region of interest, homogenize the tissue and prepare synaptosomes – isolated presynaptic terminals often attached to the postsynaptic compartment. We obtain millions of purified synaptosomes generated from specific cell types using Fluorescence-Activated Synaptosome Sorting (Biesemann et al. 2014, *EMBO J.*), a technique developed by the Herzog lab (Université de Bordeaux). This approach allows us to subsequently perform RNA sequencing, providing unprecedented insight into the transcriptomic diversity of synapse types. Profiling excitatory and inhibitory synapses revealed that our sorting procedure successfully allows us to deplete non-synaptosomal particles while enriching transcripts corresponding to our synapse type of interest. Our data confirm that excitatory and inhibitory synapses are transcriptomically distinct. In a second line of experiments, we have established a complementary primary neuronal cell culture system using the genetic mouse lines. In the future, this will allow us to induce different forms of plasticity and broadly study the transcriptomic correlates of different synapse states. Our approach reveals new insights into the transcriptomic diversity of synapse types and states. Also, it can be flexibly adapted to profile synapse remodeling during any form of plasticity and after any behavioral manipulation.

A32 - Development of a sequencing method to explore tRNA pools diversity in neuronal processes

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Neurons use diverse mechanisms to regulate and customize gene expression in axons and dendrites. The tRNA population of neurons, particularly that present in axons and dendrites is not well-described. We describe here a method for tRNA quantification in neurons by next-generation sequencing. A single-pot reaction library construction strategy and an optimized 3' oligo-adapter design for small-RNA ligation allows us to sequence low-input samples, with a current sensitivity of 10 ng RNA input. The use of TGIRT-III reverse-transcriptase, which has high processivity reading base-modified ribonucleotides, allows us to detect full-length tRNAs with strong enrichment (tRNAs represent 50-80% of all sequenced reads) and accuracy. tRNA charging status can be assessed by employing a periodate oxidation step, which removes A bases from non-aminoacylated tRNAs 3' ends; this modification can then be identified by sequencing. Among more than 600 possible isodecoders (associated with 61 anticodons isotypes) in the rat genome, we have detected ~ 250 high-confidence tRNAs in rat cortical neurons. Our method also detects differences in tRNA pools between neuronal-enriched and neuronal/glia mixed rat cortical cell cultures, revealing potential differential isodecoder expression levels for Pro-TGG/AGG, Ser-AGA, Ala-AGC and Arg-TCT tRNAs. Preliminary data also indicates that the method can detect differences in isodecoders levels between neuronal soma and dendritic compartments, allowing us to investigate the composition of neuronal localized tRNA pools.

A33 - Using single cell subcellular transcriptomics to profile the molecular diversity of compartments within neuronal circuits

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Neurons are characterized by complex morphologies that generate subcellular compartments that semi-independently sense, integrate and transmit information. Within a neuronal circuit, however, it is unclear how different neurons represent diverse information across subcellular units, which include pre- and post-synaptic compartments, dendritic and axonal segments, as well as somata and nuclei. Such representation likely relies on the molecular differentiation of single subcellular units and their transitions between different functional states. To discover the subcellular diversity within neuronal circuits, we focus on localized RNAs whose translation allow spatially and temporally defined proteome modifications. To this end, we are developing cutting edge methods that allow the high-throughput isolation of single subcellular units, and which are sufficiently sensitive to detect the minute amounts of RNAs within these compartments. First, we established a technique to separately profile the dendritic and somatic transcriptomes of single neurons by isolating compartments with laser capture microdissection and profiling the local transcriptome with scRNA-seq. We observed thousands of mRNAs in the dendrites of both glutamatergic neurons and various types of GABAergic interneurons,

including hundreds of mRNAs whose dendritic abundance varied according to cell-type. Next, we aim to characterize the RNA repertoire of single synapses using droplet microfluidics. Our approach consists of sorting and purifying synaptic particles, which are fluorescently-labelled in a cell-type specific manner. Single synaptic particles are then encapsulated into picoliter droplets containing the necessary components to convert synaptic RNAs to cDNA, adding in the process a molecular barcode unique to the RNAs of that droplet. The contents of individual droplets are then mixed and sequenced all together. Finally, by reading and sorting molecular barcodes, the RNA repertoire of individual synapses is bioinformatically reconstructed. Together, our subcellular transcriptomic approaches could allow for the characterization of individual subcellular compartments and their functional specialization within neuronal circuits of interest.

A34 - Linking cellular ultrastructure to gene expression with correlative cryo-ET/scRNA-seq

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While electron cryo-tomography (cryo-ET) can provide unprecedented information about the shape and distribution of large macromolecules within cells, it typically lacks information about cellular identity. Indeed, when examining heterogeneous cell populations, one must combine cryo-ET with specific dyes or genetic markers to identify a cell type of interest, thus greatly limiting the range of available targets. Here we developed a correlative pipeline connecting cellular cryo-ET with single-cell RNA sequencing by subsequent extraction of cryo-ET imaged cells via laser capture microdissection. We successfully imaged, extracted, and sequenced the transcriptome of individual cultured rat hippocampal neurons. The obtained sequencing results showed preservation of RNA integrity and cell health, and comparable sequencing depth and gene coverage to previous work without prior cryo-ET. Thousands of transcripts were quantified in each single cell, allowing integration and co-clustering of these imaged cells with other datasets, and their classification within glutamatergic and GABAergic neuronal subtypes. Ultimately, with this methodology we aim to enable the extraction of more information from cells, and link their cellular (ultra) structure and gene expression profiles.

A35 - Selective inhibition of synaptic protein synthesis

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Brain function and learning depend on the ability of neurons to modify their synapses with temporal and spatial selectivity. A biological process that enables this is local translation: the ability of neurons to synthesize proteins not only in the soma but also in dendrites and axons, near synapses. In order to functionally dissect the role of local protein synthesis in neuronal and brain function, we are developing

a set of tools that can inhibit protein synthesis on demand with cell-type and subcellular resolution. By controlling the expression of a ribosome-inactivating-protein with an inducible system, we previously showed that we can achieve complete and reversible protein synthesis inhibition in a cell-type of interest[1]. To differentiate the contribution of somatic versus synaptic protein synthesis, we are further engineering our tool to inhibit protein synthesis at synapses selectively. We temporarily inactivate the inhibitor with cleavable domains, and we achieve synaptic localization by fusing it to a synaptic protein. Once localized, the inhibitor can be activated by a localized protease which selectively cleaves the inactivating domains, triggering the ribosome inactivating activity locally.

Heumüller, M., Glock, C., Rangaraju, V., Biever, A., & Schuman, E. M. (2019). A genetically encodable cell-type-specific protein synthesis inhibitor. *Nature methods*, *16*(8), 699-702.

A36 - The landscape of synaptic proteome diversity

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Neurons build and diversify synaptic contacts using a plethora of protein combinations that define synaptic functional properties. The activity-dependent remodeling of the synaptic proteome modulates the efficacy of synaptic transmission and is thought to constitute the molecular correlate of learning and memory formation. More than a thousand proteins have been identified at both the pre- and postsynaptic compartment, providing substantial potential for synaptic diversity. While there is ample evidence of synaptic diversity in terms of structural types, states or functional properties, the diversity of the underlying synaptic proteomes remains largely unexplored. The specific isolation and analysis of defined synapses and their complement of proteins would enable the global investigation of synaptic proteome diversity and a detailed understanding of synapse molecular diversity would provide the missing link between the molecular architecture of a synapse and its function.

We use fluorescence-activated synaptosome sorting (FASS) to obtain highly purified synaptic terminals from genetically-identifiable synaptic populations in different brain areas and investigate cell-type and brain region specific synaptic proteomes. First, using Camk2a- and Gad2-cre driver lines we show specific purification of cortical excitatory and inhibitory synaptic terminals by FASS and quantification of the underlying synaptic proteomes using mass spectrometry-based proteomics. We then expand our analysis to determine synaptic proteome diversity in 15 distinct synapse subtypes defined by four cre-driver lines representing cell types, and five distinct brain areas. System-wide analysis of the resulting synaptic proteomes reveals novel synaptic proteins and shared as well as specialized synaptic modules. Finally, we zoom-in the synaptic proteomes of the major three subclasses of cortical inhibitory neurons, which are well characterized by distinct transcriptomic profiles, connectivity patterns and firing properties. We identify distinct synaptic proteomes for the three subtypes and highlight synaptic features relating to their functional properties.

Overall, we provide a landscape-view of cell-type and brain region-specific synaptic proteome diversity. Molecular classification of distinct synapses based on quantitative data at the synaptic protein level is the basis for a systems-level understanding of neuronal signaling in health and disease.

B1 - Directed protein evolution and engineering for the investigation of endogenous synaptic proteins

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Designing highly specific molecular tools for the investigation of endogenous protein-protein interactions (PPIs) constitutes a challenging task that can be further complicated in the context of multiple paralogs and conserved interaction surfaces. Indeed, in this case, the direct generation of selective binders or competitive inhibitors is hindered by high similarity within the evolutionary-related protein interfaces. This is the situation for the PDZ domain-mediated interactions of glutamate receptors with the synaptic scaffold proteins from the PSD-95 family. These interactions have been identified over the last decade as critical for the receptor synaptic stabilization and their function. However, the mechanisms that dynamically govern their respective synaptic retention remain poorly understood in part due to the dearth of specific tools.

To address this challenge, we have developed directed evolution and protein engineering strategies to generate innovative tools that allow us to modulate or monitor these interactions. In particular, for the modulators, we have implemented a strategy that uses a semi-rational approach to separate the modulator design into two functional parts [1]. We first achieve specificity toward a region outside of the interface by using phage display selection coupled with molecular and cellular validation. Highly selective competition is then generated by appending the more degenerate interaction peptide to contact the target interface. We apply this approach to specifically bind and block a single PDZ domain within the postsynaptic protein PSD-95 over highly similar PDZ domains in PSD-93, SAP-97 and SAP-102. In parallel, we have exploited specific binders of each PSD-95 family members to engineer fluorescent probes that can be used to directly monitor these endogenous proteins in living neurons. We also show that these tools can be easily adapted to various super-resolution imaging techniques (STED, PALM, DNA-PAINT) [2].

Our work provides the first paralog-selective set of tools to investigate endogenous PSD-95 family members, and describes a method to efficiently target other conserved PPI modules.

[1] Rimbault et al., Nat. Commun., 2019, 10, 4521.

[2] Rimbault et al., BioRxiv., 2021, doi : 10.1101/2021.04.07.438431.

B2 - Resolving postsynaptic AMPA receptor dynamics and presynaptic glutamate release during short-term plasticity

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Short-term plasticity (STP) of synaptic transmission shapes the amplitudes of synaptic responses on a timescale of milliseconds to seconds, and is considered to be important for various cognitive processes including information processing, temporal integration, and working memory. It is generally accepted that STP is expressed through activity-dependent mechanisms that scale presynaptic neurotransmitter release. However, several postsynaptic mechanisms, including AMPA receptor (AMPAR) desensitization and exchange by lateral surface diffusion, have been shown to regulate STP. Altogether, our understanding of how these pre- and postsynaptic processes collectively contribute to the expression of STP remains incomplete. Here, we generated a biotin acceptor peptide (AP) tag GluA2 knock-in mouse model, where, upon target specific expression of biotin ligase (BirAER), endogenous AP-GluA2-containing AMPAR can be immobilized on the cell surface using the tetravalent biotin binding protein NeutrAvidin (NA). Using whole-cell patch-clamp recordings to monitor postsynaptic currents and iGluSnFR 2-photon biosensor imaging to monitor glutamate release events, we found that AMPAR crosslinking decreased synaptic facilitation in Schaffer collateral CA3-CA1 synapses, while glutamate release events remained unchanged. The crosslinking-dependent decrease in facilitation was reversed by AMPAR desensitization blockers, suggesting that the modulation of STP occurs via the diffusion-dependent replacement of desensitized AMPARs in front of vesicle release sites. NA crosslinking did not impact the kinetics of AMPAR desensitization/recovery or their nanoscale organization at postsynaptic spines. We found that the respective contributions of pre- and postsynaptic mechanisms to STP expression varied among different types of synapses, suggesting that functional specializations shift the pre/post balance that defines synaptic response profiles during activity-dependent STP. We now aim to identify the relevant molecular players (e.g. AMPAR auxiliary subunits, trans-synaptic signalling cascades) and physiological processes where the regulation of AMPAR biophysics and/or surface mobility controls STP and cognitive behavior.

B3 - A synaptomic analysis reveals dopamine hub synapses in the mouse striatum

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Dopamine transmission is involved in reward processing and motor control, and its impairment plays a central role in numerous neurological disorders. Despite its strong pathophysiological relevance, the molecular and structural organization of the dopaminergic synapse remains to be established. Here, we used targeted labelling and fluorescence activated sorting to purify striatal dopaminergic synaptosomes. We provide the proteome of dopaminergic synapses with 57 proteins specifically enriched. Beyond canonical markers of dopamine neurotransmission such as dopamine biosynthetic enzymes and cognate receptors, we validated 6 proteins not previously described as enriched. Moreover, our data reveal the adhesion of dopaminergic synapses to glutamatergic, GABAergic or cholinergic synapses in structures we named "dopamine hub synapses". At glutamatergic synapses, pre- and postsynaptic markers are significantly increased upon association with dopamine synapses. Dopamine hub synapses may thus support local dopaminergic signalling, complementing volume transmission thought to be the major mechanism by which monoamines modulate network activity.

B4 - GreenT: A novel biosensor for calcium imaging in the extracellular space

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Calcium is one of the most important intracellular messengers in the brain, controlling key signaling pathways and cell functions like neuronal excitability, neurotransmitter release and synaptic plasticity. The calcium concentration is very low at rest inside cells (100 nM), but it can increase transiently to micromolar levels during Ca^{2+} influx from the extracellular space (ECS), where the concentration is more than 10,000 times higher.

While the spatio-temporal dynamics of intracellular calcium are intensely studied by fluorometry, very little is known about calcium in the ECS. This knowledge gap is mostly because existing calcium

indicators (based on small organic molecules or fluorescent proteins) have a very high calcium binding affinity, which let them detect changes in the (sub)micromolar range but render them insensitive to changes in calcium in the ECS, where they would be saturated. We report on the properties of a novel Ca^{2+} sensor designed to have an affinity in the low millimolar range. It is based on the calcium-binding protein TroponinC and mNeonGreen as fluorescent protein. The genetically encoded sensor is targeted to the plasma membrane, exposing its calcium-sensing domain to the extracellular side, where it can detect calcium specifically in the ECS.

We virally expressed the sensor in organotypic hippocampal brain slices and checked its functionality by 2-photon time-lapse imaging. Pressure-injecting or bath-applying solutions with different calcium concentrations, we characterized its sensitivity and response time to forced changes in extracellular calcium. To check the ability to report physiological changes in calcium concentration, we electrically stimulated afferent fibers (Schaffer collaterals) and looked for changes in sensor fluorescence in the CA1 target region. Unexpectedly, we detected clear signal increases after stimulation, with rise times on the order of hundreds of milliseconds and decay times of seconds. Several lines of evidence indicate that the signals reflect extrusion of calcium from active neurons and its delayed dissipation in the ECS after spontaneous and stimulated neuronal activity.

In sum, the novel sensor (GreenT) has calcium binding and fluorescent properties that make it suitable for imaging changes in extracellular calcium concentration. It is potentially a groundbreaking tool to study calcium dynamics and its regulation in the ECS.

B5 - The role of β spectrins in dendritic spines

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Recent studies using super-resolution microscopy have revealed the presence of a submembraneous actin-spectrin network known as the membrane periodic skeleton (MPS) shaping the structure of axons and dendrites. Alterations in this structure, have been implicated in neurological and psychiatric disorders such as Alzheimer's disease or schizophrenia. β spectrins are cytoskeletal proteins fundamental for the periodic organization in the axons and the somato-dendritic compartments. β spectrins link cytoskeletal and membrane protein complexes through binding sites to actin, ankyrins (Ank) and phosphoinositide lipids (PIPs) via a pleckstrin homology (PH) domain. Yet the role of these interactions in the dendritic spines and axons has been overlooked.

In our study we found that the loss of β2 spectrin in neurons is correlated to a decreased number of spines suggesting a stabilization role of β2 spectrin in the dendritic spines. Moreover, we reveal an impact of β2 spectrin in synaptic plasticity through dendritic spine morphology studies. By modulating the concentration of membrane PIPs with the use of an optogenetic tool coupled to a phosphatase we identify specific PIPs involved in β2 spectrin membrane binding. Using a combination of FRAP and single-particle tracking of β2 spectrin in live hippocampal neurons, we further show that actin, Ank and PIP lipids stabilize the β2 spectrin submembraneous anchoring and slow down its diffusion and mobility in

dendritic spines and axons. Super-resolution microscopy allowed us to visualize the nanoscale organization of β spectrins and their periodicity in neurons and show a decreased autocorrelation amplitude of β spectrins in the absence of membrane lipid and actin binding, revealing a role for PIPs and actin in the nanoscale organization of β spectrins and therefore in the regulation of the MPS.

B6 - LRRTM2 trafficking and AMPARs stabilisation at synapses

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Leucine-Rich Repeat Transmembrane (LRRTM) proteins are adhesion molecules enabling neurons to connect and communicate at synapses. Mutations in the genes encoding LRRTMs, their presynaptic partners, neurexins and their functional competitors, neuroligins, are associated with psychiatric and neurological disorders. However, molecular regulation of LRRTMs and how they structure and modulate synapse strength remain elusive. LRRTM2, the most synaptogenic member of the family is exclusively localised and enriched at excitatory synapses, where it exhibits low membrane dynamics. Interestingly, LRRTM2 is involved in synaptic transmission and plasticity, possibly through regulating surface levels of AMPARs, the main glutamatergic receptors responsible for fast transmission in the brain. In this project, we investigated the molecular mechanisms underlying LRRTM2 stabilisation and trafficking at excitatory synapses, as well as the interplay between LRRTM2 and AMPARs. We developed a structure-function approach in a novel transgenic mouse model where LRRTM2 is conditionally knocked-out during synapse formation. We demonstrated that the YxxC intracellular sequence controls LRRTM2 trafficking and confinement at synapses, independently from its PDZ-like binding motif. We then showed that synaptic AMPARs are more mobile in the absence of LRRTM2 and that the recently identified neurexin-binding site in LRRTM2 (E348Q) is required for stabilisation of synaptic AMPARs on the plasma membrane. Our results highlight LRRTM2 intracellular region as the key regulator of its membrane dynamics and confinement, while extracellular binding interfaces stabilise AMPARs at the synapse.

B7 - Selective endocytosis of Ca^{2+}-permeable AMPARs by the Alzheimer's disease risk factor CALM bidirectionally controls synaptic plasticity

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AMPA-type glutamate receptors (AMPARs) mediate fast excitatory neurotransmission, and the plastic modulation of their surface levels determines synaptic strength. AMPARs of different subunit compositions fulfill distinct roles in synaptic long-term potentiation (LTP) and depression (LTD) to enable learning. Largely unknown endocytic mechanisms mediate the subunit-selective regulation of the surface levels of GluA1-homomeric Ca^{2+}-permeable (CP) versus heteromeric Ca^{2+}-impermeable (CI) AMPARs. Here, we report that the Alzheimer's disease risk factor CALM controls the surface levels of CP-AMPARs and thereby reciprocally regulates LTP and LTD in vivo to modulate learning. We show that CALM selectively facilitates the endocytosis of ubiquitinated CP-AMPARs via a mechanism that depends on ubiquitin recognition by its ANTH domain but is independent of clathrin. Our data identify CALM and related ANTH domain–containing proteins as the core endocytic machinery that determines the surface levels of CP-AMPARs to bidirectionally control synaptic plasticity and modulate learning in the mammalian brain.

B8 – Role of presynaptic plasticity in memory encoding in CA3 hippocampal circuits: Syt7 abrogation at mossy fibers blocks short term facilitation

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The hippocampus is known to play a major role in the storage and recall of information depending on different forms of activity dependent synaptic plasticity. The connections between the DG and CA3 pyramidal cells mossy fiber synapses undergo a high dynamic range of presynaptic plasticity which endow these synaptic connections with detonator properties. The pattern of action potential firing, in the form of high

frequency bursts in the dentate gyrus strongly controls the amplitude of synaptic responses and information transfer to CA3. It is currently unknown what the role of presynaptic plasticity is at the level of CA 3 circuits in behavioural conditions.

Syt7 is calcium sensor that has been showed to be necessary for a synapse to elicit presynaptic plasticity We are using a conditional KO model for Syt7 to selectively block the expression at the presynaptic terminal of granule cells. Our work investigates the consequences of presynaptic plasticity loss at the synaptic, circuit and behavioral level. From patch clamp experiments, we demonstrate that facilitation is abrogated at mossy fiber in animals lacking Syt7 at mossy fiber. At the circuit level, we are using silicon probe simultaneous recordings in DG and CA3 to understand how the network adapts to changes in plasticity. Finally, we use the same model to describe the behavioral consequences in DG-dependent tasks. This approach will bring new understanding of the role in presynaptic plasticity which is currently not addressed in the field.

B9 - Hippocampal long-term potentiation impairment induced by Aβ42 aggregates strongly depends on the structure of the aggregate

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Among the different amyloid-β (Aβ) species involved in Alzheimer's disease (AD) pathology, Aβ42 is particularly prone to aggregation. It is Aβ42 oligomers rather than monomers or fibrils that cause the most severe impairment of synaptic plasticity and memory formation in the hippocampus in rodent models. However, recent evidence shows that oligomers arising from different stages of an aggregation reaction trigger different pathogenic cascades. Early Aβ oligomers preferentially induce membrane damage while later oligomers of the same aggregation reaction are more inflammatory and less prone to create pores. Conventional biophysical methods fail to account for these heterogeneous properties of oligomers due to their nanoscale dimensions, low abundance and short lifetime. Using single-molecule imaging techniques we characterized Aβ populations at the single-oligomer level, allowing us to take structural biophysical features into consideration when inducing chemical long-term potentiation experiments in murine organotypic hippocampal slices. To assess neuronal activity we established an optogenetic methodology combining AAV-mediated expression of a light-activated a non-selective cationic channel (CheRiff) and a calcium reporter (jRCaMP1b). Our results show that oligomers with different structural properties affect network activity in different ways. This approach will contribute to the understanding of the mechanisms by which Aβ42 oligomers induce toxic cascades resulting in memory impairment.

B10 - Physiological role of the full-length amyloid precursor protein (APP) in presynaptic plasticity and information transfer within hippocampal CA3 circuits

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The full-length amyloid precursor protein (APP), a key player in Alzheimer's disease (AD), is ubiquitously expressed throughout the brain. It is known to be abundantly expressed in presynaptic compartments where it interacts with proteins of the presynaptic release machinery[1]. However, the physiological functions of APP in the central nervous system remain elusive. Here we study the physiological role of APP in presynaptic plasticity and information transfer within the hippocampus. We focus mainly in the CA3 neuronal network since the architecture of CA3 circuits is well adapted for the fast storage and recall of information[2]. This will help understanding whether the disruption of APP functions can contribute to the pathophysiology of AD.

We deleted APP and the related protein APLP2 in selected neuronal populations of interest using a viral gene transfer strategy with specific cell promoters in APP/APLP2 double floxed mice. By combining optogenetics and *ex vivo* electrophysiology, we found that the selective deletion of APP in dentate gyrus granule cells impairs presynaptic short-term plasticity at mossy-fiber to CA3 pyramidal cells synapses, without altering the intrinsic spiking properties of granule cells. Furthermore, selective deletion of APP/APLP2 in GABAergic interneurons within CA3 influences the properties of inhibitory synaptic currents into CA3 pyramidal cells. Altogether, our data supports a role of presynaptic APP in synaptic transmission and plasticity mechanisms at identified excitatory and inhibitory synapses, and thus sheds light on the physiological contribution of full-length APP to hippocampal circuit activity.

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B11 - Astrocytic EphB3 receptors control NMDA receptor functions and memory

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Long-term synaptic plasticity is considered as the cellular substrate of learning and memory. This includes long-term synaptic potentiation (LTP), which corresponds to a persistent increase of synaptic

transmission. The most common form of LTP depends on glutamatergic NMDA receptors whose activation requires the binding of glutamate and of a co-agonist that was identified to be D-serine at hippocampal CA3-CA1 synapses. Our previous work in the hypothalamus has indicated that the close apposition of the astrocytic processes with synaptic neuronal elements was essential for the availability of D-serine in the synaptic cleft. Interestingly, activation of cell adhesion molecules (CAMs) depends on the close apposition of two membrane elements, making them very likely to participate in this glia-neuron interaction process. Data obtained in astrocytic cultures have shown that the release of D-serine was induced by the activation of astrocytic EphB3 receptors. However, we do not know whether this requires the interaction of the astrocytic EphB3 receptor with its neuronal synaptic partner and whether it could impact NMDAR functions. Here, we first established that the activation of EphB3 receptors in acute hippocampal slices from the brain of adult mice led to an increase of D-serine availability at CA3-CA1 synapses, inducing an increase of NMDAR activity. Then, selective inhibition of astrocytic EphB3 using a viral approach impaired the induction of LTP as well as novel object recognition memory. Importantly, exogenous application of D-serine was able to rescue LTP and memory deficits. Altogether, our data indicate that astrocytic EphB3 receptors play a key role in play a key role in NMDAR functions.

B12 - Cell-type specific mechanisms of neuropeptide secretion

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Neuropeptide release from dense-core vesicles (DCVs) is of critical importance in the nervous system, due to the regulatory role of neuropeptides in processes such as synaptic plasticity, circadian rhythm, and emotions. A few proteins have been shown to specifically regulate neuropeptide secretion in the brain, including the GTP-binding protein Rab3. Absence of all four Rab3 genes completely blocks neuropeptide secretion, while synaptic vesicle exocytosis is only mildly affected[1,2]. However, it is unknown whether this phenotype is conserved across different neuronal cell types, and if all four Rab3 paralogs play a role in neuropeptide secretion. Here, we identify Rab3a as the key paralog enabling neuropeptide secretion in hippocampal neurons, which are predominantly glutamatergic. Interestingly, Rab3a is dispensable for DCV exocytosis in striatal neurons, which are exclusively GABAergic. Inhibitory hippocampal neurons also display normal neuropeptide secretion in absence of Rab3a. Neuropeptide secretion is only mildly affected in striatal neurons lacking all four Rab3 paralogs, showing a striking difference with earlier data using hippocampal neurons. We conclude that Rab3a is the main regulator of neuropeptide secretion in glutamatergic neurons. Moreover, GABAergic cells display Rab3-independent DCV fusion, showing that neuropeptide secretion mechanisms are cell-type specific.

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B13 - Synaptic compensation and regeneration in Alzheimer's disease

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Synaptic loss is one the most prominent features of Alzheimer's disease (AD) as it strongly correlates with cognitive deficits. Although much is known about the mechanisms underlying synaptic loss, the compensatory mechanisms implemented by the brain to counteract synaptic loss remain largely unexplored.

The main aim of this project is to investigate the synaptic compensatory mechanisms counteracting synaptic loss in AD and consequently, delaying the onset of cognitive deficits.

One of the reasons we know so little about synaptic compensation in AD is probably our inability to predict which synapses will be lost and as such, which ones will be compensated. To overcome this limitation, we developed an optogenetic tool to artificially eliminate synapses with high spatiotemporal control to model this feature of AD in order to monitor the emergence of structural and functional synaptic compensation over time. Using this targeted approach, we recently discovered that the artificial elimination of synapses is compensated by the early enlargement of surviving synapses (24hrs) and the later regrowth of synapses (>1week) both in organotypic hippocampal slice cultures and in in-vivo in the primary visual cortex. In addition, we are currently validating these findings both in in-vitro and in-vivo models of Alzheimer's disease.

We believe that modelling synaptic compensation in Alzheimer's disease is the first step toward understanding the molecular mechanisms underlying these inherently protective synaptic compensatory events, which may be crucial for delaying the onset of cognitive deficits in AD.

B14 - Correlating STED and synchrotron XRF nano-imaging to explore metal functions in synapses

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Zinc and copper are involved in neuronal differentiation and synaptic plasticity but the molecular mechanisms behind these processes are still elusive due in part to the difficulty of imaging trace metals

together with proteins at the synaptic level. We correlate stimulated-emission-depletion microscopy (STED) of proteins and synchrotron X-ray fluorescence (XRF) imaging of trace metals, both performed with 40 nm spatial resolution, on primary rat hippocampal neurons [1]. DIV15 neurons were cultured on silicon nitride membranes and labeled with silicone rhodamine fluorogenic probes, SiR-tubulin and SiR700-actin. Synchrotron XRF was performed on the ID16A Nano-Imaging beamline at the European Synchrotron Radiation Facility (ESRF), to map element distributions in dendrites and dendritic spines. Synchrotron radiation X-ray phase contrast imaging (PCI) was carried out as well to map the structural density with 15 nm spatial resolution. STED microscopy was performed on a Leica DMI6000 TCS SP8 X microscope at the Bordeaux Imaging Center (BIC).

Thanks to this experimental setup, we reveal the co-localization at the nanoscale of zinc and tubulin in dendrites with a molecular ratio of about one zinc atom per tubulin-αβ dimer. We observe the co-segregation of copper and F-actin within the nano-architecture of dendritic protrusions. These results indicate new functions for zinc and copper in the modulation of the synaptic cytoskeleton morphology, a mechanism associated to structural plasticity. From a methodological perspective, the combination of STED super-resolution microscopy and nano-SXRF imaging stands as a solid new tool for the identification of metalloproteins directly in cells by correlating cellular imaging methods at a supramolecular scale.

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B15 - Investigating synapse formation and nano-organization with advanced 2D in vitro models

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Information transfer in the brain is ensured by a complex network of specialized interconnected neurons responding to environmental cues. The proper functioning of this network relies on the establishment and maintenance of synaptic connections, the nanoscale organization of their molecular components, and the plasticity of their architecture. To better understand synapse development and characterize its molecular organization, we use single molecule STORM in combination with a homebuilt micropatterning platform LIMAP [1], now commercialized by the company ©Alveole. Micropatterning of environmental cues such as cell adhesion proteins, allows us to generate thousands of standardized hemi synapses from primary neurons [2] on which we can study the signaling events involved in synapse formation. Using STORM microscopy, we can precisely locate patches of synaptic markers and cytoskeleton [3] elements within these micropatterned areas to model an average structural organization of synapse components. Our aim is to model the development of synapses over time by characterizing the sequence of events leading from the establishment of an initial contact to the

formation of stable synapses. We anticipate that by controlling the shape and size of individual spines with the micropatterns, we will be able to average together super-resolution images from hundreds of spines and thus get new insights, at the nanoscale, on the mechanisms of pre and post synapse alignment. We also hope to get a better understanding of how subclasses of adhesion proteins such as cadherins interact with the cytoskeleton to shape dendritic spines while others induce functional synapse formation aligning the presynaptic active zone of axonal boutons with post synaptic densities.

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B16 - Excitatory glycine GluN1/GluN3A NMDA receptors in the adult hippocampus

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The unconventional glycine-binding GluN3A subunit confers to N-methyl-D-aspartate (NMDA) receptors atypical biophysical and signaling properties. GluN3A containing NMDARs are highly expressed during postnatal development, when they are thought to modulate synapse maturation and stabilization. GluN3A expression declines in the transition to adulthood but is maintained at significant levels in several specific brain areas. Recent evidence suggests that GluN3A operates in the adult brain by associating with the glycine-binding subunit GluN1, to form excitatory GluN1/GluN3A receptors activated by glycine only (eGlyRs) which control neuronal excitability and emotional behaviors. Here we investigate eGlyRs in the adult mouse hippocampus and disclose regional differences, cell specificity and endogenous mode of activation with the purpose of unveiling eGlyR cellular physiology and implication in behavior. We show that eGlyRs reside in specific neuronal populations including CA1 pyramidal cells and SST-positive interneurons but not CA3 pyramidal cells and granule cells of the dentate gyrus. Within CA1 pyramidal neurons, eGlyR functional expression follows a marked dorso-ventral gradient, with no expression in the dorsal region but high expression in the ventral one. Unlike CA1 pyramidal neurons, SST-positive interneurons express eGlyRs in both the dorsal and the ventral region. eGlyRs are tonically active, sensing endogenous glycine levels and controlling neuronal excitability in pyramidal cells and SST-positive interneurons. Thus, eGlyRs establish a signaling system in the adult hippocampus with

unique functional properties and expression that strikingly differ from conventional NMDARs. Our work opens new perspectives on the exploration of eGlyRs in hippocampal function with an emphasis on ventral hippocampus related behaviors.

B17 - Electrophysiological characterization of cognition-relevant brain oscillations in a radiotherapy mouse model

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Brain irradiation has been proven to cause long-term neurocognitive complications that usually involve memory and learning problems. Gamma oscillations in the hippocampal formation play a key role in memory and learning processes. Gamma oscillations (30-80Hz) are rhythmic fluctuations in local field potentials that result from the synchronization of action potential generation in excitatory pyramidal cells and inhibitory GABAergic interneurons, in particular fast-spiking interneurons. Alterations in the synchronous activity of pyramidal cells and fast spiking interneurons are related to cognitive deficits and neurodegenerative diseases. Our aim was to determine the effect of brain radiotherapy on cognition-relevant hippocampal oscillations. For this purpose, we performed local field potential and patch clamp recordings in acute hippocampal slices of sham (SH) and 8 Gy irradiated (IR) mice 2 weeks, 6 weeks, and 6 months after cranial irradiation. Here we show that gamma oscillations and the neuronal synchrony responsible for their generation were compromised 6 weeks after irradiation. At this timepoint, spike-gamma coupling of both IR fast spiking interneurons and pyramidal cells was impaired. In contrast, there was no significant difference in spike-gamma coupling of fast spiking interneurons and pyramidal cells between SH or IR mice 6 months after irradiation. Gamma oscillations were still impaired at this late timepoint, although to a lesser extent. These observations suggest a recovery of the inhibition/excitation balance in the neuronal network 6 months after irradiation. Taken together, our results demonstrate that, while irradiation did not have an evident effect on gamma oscillations and neuronal activity as early as 2 weeks after radiation exposure, a radiation-induced impairment became evident after 6 weeks and was still present, although to a lesser extent, 6 months after irradiation. These findings suggest that exposure to cranial irradiation at the dosage employed in this study has a delayed detrimental effect on gamma oscillations and the underlying neuronal activity. The mechanisms leading to this impairment of gamma oscillations remain to be elucidated.

B18 - Novel Optogenetic tools for the Spatio-Temporal Photocontrol of AMPARs mobility

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The dynamic regulation of AMPA glutamate receptors (AMPARs) trafficking into and out of the synapse is a major mechanism underlying synaptic plasticity, a fundamental basis of learning and memory. However, the detailed molecular mechanisms governing the dynamics of these postsynaptic receptors are still not fully understood, stressing the need for new methods to modulate and monitor these processes. We have developed innovative approaches based on the use of light-sensitive groups to stabilize or destabilize the AMPARS at the membrane level.

On one hand, we have designed Photoactivatable competing ligands to destabilize AMPARs by disruption of the natural interaction between Stargazin and PSD-95. On the other hand, we have engineered photoswitchable crosslinkers to specifically recognize AMPAR subunits and control with spatio-temporal resolution the mobility and local number of AMPARs.

The design and production of these optotools have involved molecular/cell biology, protein engineering and biophysical characterization, for later on, validated them in heterologous cells by Advanced Fluorescence Techniques. In order to characterize these approaches in transfected eukaryotic cells as a model of complex cellular context, a new red shifted FRET pair has been developed to monitor protein interactions. Besides heterologous cells, the approaches have been also validated by u-PAINT in neurons.

The approaches here engineered should most importantly allow tackling fundamental questions related to AMPARs mobility but also be of high impact on a large scientific community as they could be transferable to many systems.

B19 - Super-resolution analysis of the nano-anatomical determinants of the function and plasticity of dendritic spines

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The essence of synapses is to mediate rapid and flexible electrochemical communication between neurons in the brain. Despite intense research, we still have a poor understanding of the biophysical mechanisms that shape the spatial and temporal integration of electrical signals in dendritic spines and the parent dendrite. For instance, we don’t know if the spine neck (a prominent physical constriction

between the spine head and dendrite) could exert enough of an electrical resistance to significantly influence the voltage transformations in the spine head, dendrite and cell body. Moreover, it remains completely unknown if structural changes in spine neck geometry could be a physiological mechanism to regulate synaptic strength.

To address these fundamental questions, we apply an array of optical and neurophysiological techniques, variably combining patch-clamp electrophysiology, live-cell STED imaging, 2-photon glutamate uncaging and voltage imaging in organotypic hippocampal brain slices.

We are implementing two approaches to estimate the amplitude of excitatory synaptic potentials in the spine head and its attenuation across the neck, either based on indirect electrophysiological measurements or direct optical recordings of voltage signals. For both approaches, we use 2-photon glutamate uncaging to controllably activate glutamate receptors on the postsynaptic membrane. The first approach is based on recording the currents and voltages for a given strength of glutamate uncaging, which allows us to decouple synaptic conductance and input resistance. By modeling the elicited voltages measured in the soma, it is possible to estimate the amplitude of the voltage reached in the spine head. We carry out this sequential voltage/current clamp analysis in spines of varying neck geometry (thin, long, wide, short as ascertained by STED microscopy), seeking to establish a correlation between spine voltage and nanoscale neck structure. In the second approach, we will optically measure the synaptic voltages and their attenuation in the dendrite using recent genetically encoded voltage sensors. Likewise, we will carry out the optical approach in a morphologically diverse population of spines, to compare and validate the measurements with the electrophysiological approach.

In a second step, we will apply these two approaches before and after the induction of structural plasticity by repetitive 2-photon glutamate uncaging using established protocols. This will allow us to address the long-standing question of whether structural plasticity actually has appreciable or even important electrical consequences on synaptic function beyond providing for larger spine heads to accommodate more synaptic receptors.

B20 - Spatial regulation of coordinated excitatory and inhibitory synaptic plasticity at dendritic synapses

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Traditionally, the flexibility of neuronal network was thought to mainly rely on the potentiation and depression of excitatory synapses. However, recent evidences revealed several types of inhibitory plasticity, raising the important question of how GABAergic and glutamatergic plasticity are coordinated during neuronal activity. Here, we characterized a non-Hebbian form of inhibitory postsynaptic potentiation (iLTP) induced by postsynaptic depolarizations of hippocampal principal cells. Interestingly, the same protocol induced depression at glutamatergic synapses (LTD), thus indicating an anti-

homeostatic relation between inhibitory and excitatory plasticity. Photolysis of caged glutamate or caged GABA revealed that the aforementioned glutamatergic LTD and GABAergic iLTP are expressed postsynaptically. Subsequently, we investigated how synaptic plasticity induced at individual glutamatergic spines affects the strength of neighboring GABAergic synapses. To this end we induced "single spine LTP" by pairing the postsynaptic depolarizations with repetitive glutamate uncaging at individual spines while simultaneously measuring the strength of adjacent dendritic GABAergic synapses by GABA uncaging. Interestingly, we found that, following the delivery of this hebbian-like protocol, GABAergic synapses located within 3 μm from a stimulated spine showed depression (iLTD), while further synapses still showed iLTP. This spatial dependent reversion from iLTP to iLTD of inhibitory plasticity induced by heterosynaptic plasticity from spines was dependent on calcium influx through L-type voltage gated calcium channels and the activation of the protease calpain. Our findings indicate that changes of calcium concentration can finely coordinate both glutamatergic and GABAergic synaptic plasticity at dendritic level suggesting that the dendritic E/I balance are selectively tuned in spatially restricted dendritic sub-regions.

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B21 - Inducing an engram using two-photon holography

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Since Hebb (1949) it has been hypothesised that memories are formed by the plasticity of synaptic connections between neurons, however this is yet to be clearly demonstrated experimentally from memory induction through to behaviour. In the first part of this project, a protocol has been developed to induce a short-term memory in *ex vivo* brains using two photon holography and two photon calcium imaging. This project uses the brains of *Drosophila* larvae so that in the future we can computationally reconstruct the specific neural circuit used for this memory at both the synaptic and molecular levels, using SEM (scanning electron microscopy) and TEM (transmission electron microscopy), respectively. So far, we have shown that changes in calcium signalling can be seen from the beginning to the end of the

optical short-term memory protocol which can be directly related to the known behavior of the larva following appetitive odour conditioning. Therefore we are able to induce a memory engram into the brain and in the future, determine how this is physically stored through molecular, synaptic or cellular mechanisms.

B22 - Identification of synapse diversity in the neocortex using single cell transcriptomics reveals selective synapse signatures

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Synaptic diversity has been described from different perspectives, ranging from the specific neurotransmitters released, to their diverse biophysical properties and proteome profiles. However, synapse diversity at the transcriptional level has not been mapped systematically for all synapse populations in the brain. Here, we identify the synaptic diversity across neuronal cell types in the mouse neocortex using single cell transcriptomics. We quantified the importance of specific synaptic features for neuronal identity by means of combining the SynGO (Synaptic Gene Ontology) database and machine learning algorithms. We found that synaptic genes contain information on cell type diversity similarly to all expressed genes. Additionally, we observed that cell type encoding is not only present within the whole synaptic transcriptome. Conversely, both pre- and post- synaptic functions and cellular components (described in SynGO) specifically allow discrimination between different cell types. By inspecting the gene correlation network underlying different cell types, we identified gene modules comprising three different types of synaptic diversity: gradient expression, gradient expression in selected cell types and cell class or type specific profiles. These unique synaptic gene sets provide a deeper understanding of synapse diversity in the neocortex and are potential markers to selectively identify populations of synapses derived from specific neuronal populations.

B23 - Protein Tyrosine sulfation in synaptic plasticity and memory

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Post-translational modifications (PTM) of proteins play important roles in learning and memory. Protein tyrosine-O-sulfation is a common post-translational modification in eukaryotes both animals and plants (Moore et al 2003, Corbeil et al 2005). Tyrosine-O-sulfated proteins existing throughout the metazoan evolution, appear to be absent in unicellular eukaryotes and prokaryotic organisms (Moore et al 2003). Several tyrosine-O-sulfated proteins have been shown to play important roles in different physiological processes (Kanan et al., 2013). In addition, protein tyrosine sulfation helps in the proper development of retinal structure and function (Sherry et al., 2010). In the brain also, tyrosine sulfation seems to play an important role. The developmental pattern of TPSTs suggests that protein tyrosine sulfation plays a crucial role in embryogenesis and neuronal maturation. HS helps in neurite growth promoting activity from the surface of neocortical explants and the neurite outgrowth was decreased in presence of sodium chlorate. Sulfation regulates neural precursor cell proliferation and differentiation in the developing mouse spinal cord. However, the role of protein tyrosine-O-sulfation in synaptic plasticity and memory is not understood. Here our study reveals that sulfation contributes to long-term potentiation in the hippocampal slices. Sulfation inhibition impairs long-term spatial memory without affecting task learning and short-term memory. Further, the LTP-inducing stimulus increases protein tyrosine sulfation. These results suggest a vital role of tyrosine sulfation in LTP and memory.

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B24 - Circuit-specific synaptic and behavioral impact of GluA1 CTD deletion

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AMPA receptors (AMPAR) are ionotropic glutamate receptors that mediate fast excitatory synaptic transmission. Several forms of synaptic plasticity including long-term potentiation (LTP) rely on the regulation of synaptic AMPAR content. GluA1 is one of four pore-forming subunits that comprise the

AMPAR and is essential for LTP. The GluA1 carboxy-terminal domain (CTD) has long been implicated in receptor trafficking, but whether it plays a specific role in certain synaptic plasticity phenomena and /or brain circuits remains unclear. We and others previously showed that the GluA1 CTD is not required for AMPAR mediated synaptic transmission and LTP at hippocampal CA3-CA1 synapses. The present study aims to dissect the functional role of the GluA1 CTD in synaptic transmission in other synapse types and its impact in GluA1-dependent behavior. To this end, we utilized our recently developed HA-ΔCTD GluA1 KI mice and combined behavioral, histological and electrophysiology approaches. Interestingly, we found that ΔCTD GluA1 KI mice share some, but not all of the behavioral phenotypes of GluA1 KO mice. Specifically, GluA1 CTD truncation led to i) increased novelty-induced hyperlocomotion, ii) increased time spent in open arms in an elevated plus maze, and iii) impaired fear expression but strikingly intact fear memory after contextual fear conditioning. Expression of the immediate early gene *c-fos* was analyzed to identify potential region-specific changes in neuronal activity in HA-ΔCTD GluA1 KI mice. We found that HA-ΔCTD GluA1 KI mice had exacerbated novelty-induced *c-fos* immunoreactivity in dentate gyrus and CA3, but not CA1. Our preliminary observations using whole-cell patch-clamp recordings in hippocampal acute slices from young (3-6 week-old) and adult (6 months old) HA-ΔCTD GluA1 mice of either sex indicate that, while AMPA trafficking and LTP are unaffected in CA1, synaptic transmission is altered in HA-ΔCTD GluA1 DG granule cells. Ongoing experiments will determine whether GluA1 CTD deletion affects LTP expression in DG and other brain regions. Future research will explore the molecular mechanisms whereby the GluA1 CTD domain may interact with scaffolding proteins to regulate synaptic AMPAR trafficking in a region-specific manner. Our findings suggest that the GluA1 CTD plays a synapse type-specific role in AMPAR synaptic transmission, thereby modulating some GluA1-dependent synaptic plasticity mechanisms underlying specific behaviors.

B25 - LRRTMs mediate context-dependent synapse organization and plasticity in developing and mature hippocampal circuits

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LRRTMs are postsynaptic cell adhesion proteins that have region-restricted expression in the brain. To determine their role in the molecular organization of synapses in vivo, we studied synapse development and plasticity in hippocampal neuronal circuits in mice lacking both *Lrrtm1* and *Lrrtm2*. We found that LRRTM1 and LRRTM2 regulate the density and morphological integrity of excitatory synapses on CA1 pyramidal neurons in the developing brain but are not essential for these roles in the mature circuit. Further, they are required for long-term-potentiation in the CA3-CA1 pathway and the dentate gyrus, and for enduring fear memory in both the developing and mature brain. Our data show that LRRTM1 and LRRTM2 regulate synapse development and function in a cell-type and developmental-stage-specific manner, and thereby contribute to the fine-tuning of hippocampal circuit connectivity and plasticity.

B26 - The cyclase-associated protein 2 controls Cofilin-actin rods formation in Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by Amyloid β(Aβ)-driven synaptic dysfunction in the early phases of pathogenesis. In addition to spine loss, cytoskeletal abnormalities, such as cofilin-actin rods, have been reported in AD patients and animal models [1]. Cytoplasmic rods contain cofilin and actin and are formed upon exposure to different stressors, including Aβ oligomers [2]. We have recently demonstrated that the actin-binding protein Cyclase-associated protein 2 (CAP2) is a master regulator of cofilin localization and activity, through the Cys32-dependent CAP2 dimerization. These mechanisms are altered in AD, suggesting an involvement of CAP2/cofilin pathway in AD pathogenesis [3]. This work aims to explore the involvement of CAP2 in Aβ-induced actin rods formation during the early phases of AD pathogenesis taking advantage from an in vitro and an in vivo model of the disease. Firstly, we demonstrated that Aβ oligomers impair CAP2/cofilin pathway both after a short exposure and when cofilin-actin rods are generated, although the resulting effects are different. Then, we found that CAP2 accumulates in actin rods, when specifically induced by Aβ exposure, but not when neurons are exposed to a different stressor. Finally, we show that CAP2 overexpression can prevent rods formation and spine loss, through a mechanism that requires CAP2 capability to form Cys32-dependent dimers. Overall, our data support the involvement of cofilin/CAP2 cooperation in different biological aspects of AD pathogenesis in neuronal cells, thus providing novel potential therapeutic target for AD.

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B27 - MDGAs are fast-diffusing molecules that delay excitatory synapse development by altering neuroligin behavior

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MDGA molecules can bind neuroligins and interfere with trans-synaptic interactions to neurexins, thereby impairing synapse development. However, the sub-cellular localization and dynamics of MDGAs, or their specific action mode in neurons remain unclear. Here, surface immunostaining of endogenous MDGAs and single molecule tracking of recombinant MDGAs in dissociated hippocampal neurons reveal that MDGAs are homogeneously distributed and exhibit fast membrane diffusion, with a small reduction in mobility across neuronal maturation. Knocking-down/out MDGAs using shRNAs and CRISPR/Cas9 strategies increases the density of excitatory synapses, the membrane confinement of neuroligin-1, and the phospho-tyrosine level of neuroligins associated with excitatory post-synaptic differentiation. Finally, MDGA silencing reduces the mobility of AMPA receptors, increases the frequency of miniature EPSCs (but not IPSCs), and selectively enhances evoked AMPA-receptor mediated EPSCs in CA1 pyramidal neurons. Overall, our results support a mechanism by which interactions between MDGAs and neuroligin-1 delays the assembly of functional excitatory synapses containing AMPA receptors.

Full reference: Toledo et al. eLife. 2022 May 9; 11:e75233.

B28 - GRID1/GluD1 homozygous variants linked to intellectual disability and spastic paraplegia impair mGlu1/5 receptor signaling and excitatory synapses

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The ionotropic glutamate delta receptor GluD1, encoded by the GRID1 gene on chromosome 10, is involved in synapse formation, function, and plasticity. GluD1 does not bind glutamate, but instead cerebellin and D-serine, which allow the formation of trans-synaptic bridges, and trigger metabotropic signaling. Though widely expressed in the nervous system, pathogenic mutations of GluD1 have not been characterized in humans so far. We report homozygous missense GRID1 mutations in siblings from two consanguineous families presenting with intellectual disability and spastic paraplegia, without or with glaucoma, a threefold phenotypic association whose genetic bases had not been elucidated previously. Molecular modeling indicated that one mutation alters cooperativity between cerebellin and D-serine binding GluD1 domains, whereas the other mutation alters D-serine binding. Expression, trafficking, physical interaction with metabotropic glutamate receptor mGlu1, and cerebellin binding of GluD1 mutants were not conspicuously altered. Conversely, we found that GluD1 mutants hampered signaling of metabotropic glutamate receptor mGlu1/5 via the ERK pathway in primary cortical cell culture. Moreover, both mutants impaired dendrite morphology and excitatory synapse density in neurons of primary hippocampal culture. We have developed a new mouse model harboring one of these mutation in the GRID1 locus and started characterizing synaptic transmission in the CA1 area of the hippocampus. Results indicate alteration of excitatory but not inhibitory transmission and a change in the composition of ionotropic glutamate receptors consistent with the synaptic organizer role of GluD1. These results show that the clinical phenotypes are distinct entities segregating in the families as an autosomal recessive trait, and caused by pathophysiological effects of GluD1 mutants involving metabotropic glutamate receptor signaling and neuronal connectivity. Our findings unravel the importance of the GluD1 receptor signaling in sensory, cognitive and motor functions of the human nervous system.

B29 - Excitatory synaptogenesis in the anterior cingulate cortex is required for effort control

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Synaptogenesis is essential for circuit development; however, it is unknown whether it is critical in adulthood for learning and performing voluntary behaviors. Here we show that reward-based training in mice induces excitatory synapse formation onto Anterior Cingulate Cortex neurons projecting to the dorsomedial striatum ($ACC_{->DMS}$). We used germline and conditional knockout mice for Gabapentin/Thrombospondin receptor α2δ-1 required for excitatory synaptogenesis in the cortex and found that loss of α2δ-1 in the adult $ACC_{->DMS}$ circuit is sufficient to reduce training-induced excitatory synaptogenesis. Surprisingly, this manipulation did not affect learning, instead caused a profound increase in effort exertion. Furthermore, optogenetic inhibition and activation of $ACC_{->DMS}$ neurons were sufficient to modulate effort exertion in wild-type mice and rescued the increased effort exertion of the conditional α2δ-1 mutants. These results highlight the importance of synaptogenic signaling in adults and pinpoint the $ACC_{->DMS}$ neuronal circuit as the controller of effort exertion during voluntary behaviors.

B30 - Plasticity mechanisms for maternal oxytocin release in response to infant cues

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In my newly established lab, we are specifically interested in understanding the synaptic and circuit mechanisms in the maternal brain which underlie the neural processing of cues from the newborn to promote hormonal release and parental behaviors. Becoming a mother is a powerful transformation

affecting different brain and body functions. Critical adaptations during the postpartum period influence maternal hormonal release to maintain lactation and infant care. One important molecular signal for the maternal brain is oxytocin (OT), a nine amino acid peptide produced mainly in the hypothalamus (Valtcheva 2019). OT is believed to powerfully enhance parental behaviors by increasing the salience of sensory cues from the offspring (Grinevich 2018). However, it remains unexplored what sensory cues from infants can activate OT neurons in new mothers. Here we describe a neural circuit routing auditory information about infant vocalizations to the OT system in maternal mice (dams). By performing in vivo cell-attached and whole-cell recordings from optically-identified OT neurons, as well as fiber photometry in awake dams, we found that OT neurons, but not other hypothalamic cells, are activated following playback of pup distress vocalizations. Using anatomical tracings and channelrhodopsin-assisted circuit mapping, we identified the projections and brain areas (from inferior colliculus and auditory cortex to posterior intralaminar thalamus, PIL) relaying auditory information to OT neurons. In hypothalamic brain slices, we found that optogenetic activation of PIL fibers led to long-term depression of synaptic inhibition in OT neurons mediated by postsynaptic internalization of GABAARs via dynamin signaling. This plasticity was occluded if dams were exposed to pup calls prior to plasticity experiments in brain slices. Using a genetically-encoded OT sensor (Mignocchi et al., 2020), we demonstrated that pup calls, but not pure tones, were efficient in triggering central OT release in downstream areas such as the VTA, and that this was dependent on the activation of PIL to PVN pathway. Finally, inhibiting PIL projections to hypothalamus with chemogenetics, perturbed pup retrieval behavior in dams by decreasing the amount of pups retrieved and increasing the latency of retrieval over multiple trials. These findings suggest that the thalamus-hypothalamus noncanonical auditory pathway may be a specific circuit for the detection of social sounds, important for disinhibiting OT neurons, gating OT release in downstream brain areas, and sustaining maternal performance over time. We are currently investigating the contribution of different inputs and hypothalamic astrocytes in gating synaptic plasticity of OT cells and the processing of infant cues (vocalizations, suckling).

B31 - Role of the presynaptic SV2A protein in the control of excitation/inhibition (E/I) balance in hippocampal CA3 circuits

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The presynaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam and a widely used tomography synaptic marker. Despite being known since 1985 and being ubiquitously expressed in the brain, the function of SV2A as well as its effect in circuit dynamics has remained rather elusive. Constitutive SV2A knock-out mice display severe seizures and death after a few weeks, and mutation of the protein in humans can lead to epilepsy. We have examined the electrophysiological consequences of deleting SV2A from hippocampal mossy fibers. For this, we have used SV2A-conditional KO mice in combination with viral gene transfer to allow for expression of the Cre recombinase and an optogenetic tool (ChIEF) in dentate gyrus granule cells (GC). The deletion of SV2A leads to a decreased excitability in granule cells, but does not affect action potential properties. We took advantage of this

lack of change in action potential to study the changes in synaptic currents from onto CA3 pyramidal cells. Whereas the excitation of pyramidal cells was unchanged, the feed-forward inhibition was increased during bursts of activity. This increased inhibition led to a decreased E/I ratio on the principal output cells of the CA3. Overall, we show how the absence of SV2A leads to an increased inhibition of CA3 pyramidal cells, not by directly affecting inhibitory transmission but by increasing excitation of interneurons, intensifying feed-forward inhibition from DG to CA3.

B32 - Ribbon precursor dynamics during synapse assembly in cochlear inner hair cells

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Sensory inner hair cells (IHC) of the mammalian cochlea are mechanoreceptive cells that transform sound waves into neural code at their highly specialized ribbon-type synapses with afferent spiral ganglion neurons. Ribbons are multi-protein scaffolds within the cytomatrix of the active zone (AZ) and mainly comprised of the structural protein RIBEYE. By ensuring rapid synaptic vesicle (SV) replenishment, ribbons enable the indefatigable and temporally accurate encoding of auditory stimuli. During synapse assembly and subsequent maturation, ribbon size and the ribbon-tethered SV pool gradually increases – a process that is at least in part mediated by the translocation of floating cytosolic ribbon precursors towards developing AZs, where they likely fuse with already membrane-anchored ribbons. Although assumed to be a dynamic process, the molecular pathways underlying the developmental assembly of IHC ribbon-type AZs have remained largely elusive thus far. While findings in other ribbon-bearing sensory systems suggest synaptic ribbons to function as an activity-adaptive scaffold, real-time *in situ* analysis of putative IHC ribbon plasticity is still lacking to date. To now address this shortcoming, we devised an organ of Corti organotypic culture-based model system to conduct adeno-associated virus (AAV)- mediated labeling of IHC ribbons in context of pre- and postsynaptic structures. Virally-transduced cultures were used to perform long-term, multi-color live-cell imaging experiments to monitor ribbon synapse assembly and activity-dependent structural and positional plasticity at the presynaptic AZ of developing IHCs.

In this study, we employed a comprehensive methodological approach combining spinning disk confocal and volumetric multi-color widefield microscopy that enables close to real time acquisition speed with pharmacological manipulation. Using this approach, we investigated the mobility of membrane-anchored and membrane-proximal ribbons over various timespans as well as activity states and extracted information on precursor morphology, velocity, trajectory and directionality. Moreover, we used super-resolution microscopy to assess the molecular composition of free-floating ribbon precursors. We found that IHC ribbon precursors display a high degree of mobility and a highly adaptive morphology, which strongly depended on its state of engagement with the synaptic context. Moreover, ribbon precursor displacement and volume appeared to be affected by distinct states of spontaneous IHC activity. Finally, morphological plasticity of synaptic ribbons during development does not follow a unidirectional trajectory towards the AZ, but appears reversible and highly dynamic. In summary, our data indicate that developmental spontaneous activity is a key regulator of ribbon morphology and may hence influence synaptic assembly and functional properties of maturing IHCs.

B33 - Developmental regulation of homeostatic plasticity in mouse primary visual cortex

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Neural circuits need to maintain overall stability in the face of ongoing perturbations and to ensure the fidelity of information transfer and storage during learning and development. This is achieved by a set of homeostatic plasticity mechanisms that adjust excitation, inhibition, and intrinsic excitability. A major information gap in the field is how these distinct forms of mechanisms are regulated during development, mainly due to the lack of a uniform paradigm that allows for chronic activity manipulation at different developmental stages. To overcome this limitation, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to directly suppress neuronal activity of pyramidal neurons in layer (L) 2/3 of mouse primary visual cortex (V1) at two developmental timepoints: the classic visual system critical period (CP) and early adulthood. We show that 24 hours of DREADD-mediated activity suppression is sufficient to simultaneously induce excitatory scaling up and intrinsic homeostatic plasticity in L2/3 pyramidal neurons during the CP. Importantly, DREADD-mediated homeostatic mechanisms show similar molecular characteristics as those induced pharmacologically or by visual deprivation. We next repeated the same paradigm in adult mice. Surprisingly, while excitatory synaptic scaling persisted into adulthood, intrinsic homeostatic plasticity was absent. Instead, we found that these adult neurons homeostatically adjusted quantal inhibitory inputs, which was absent in their younger counterparts. Therefore, the same population of neurons can express distinct sets of homeostatic plasticity mechanisms at different development stages. Our findings suggest that individual homeostatic plasticity mechanisms can be recruited in a modular manner, and can be turned on and off to meet the evolving developmental needs.

B34 - The disease-associated synaptic protein syngap is involved in Ampa receptor organization

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The formation of the synapse as well as their strengthening or pruning is a crucial process during neurodevelopment. Many different proteins are involved in this process and their spatiotemporal expression is finely tuned. Disturbance of this balance due to loss-of-function mutations can have drastic effects on synaptic function and circuit development. The SYNGAP1 gene encodes the synaptic Ras/Rap GTPase-activating protein (synGAP) which is one of the most abundant proteins in the postsynaptic density (PSD) of excitatory synapses. SynGAP interacts with PSD-95, transmembrane AMPAR regulatory proteins (TARPs) and N-methyl-D-aspartate (NMDA) receptors at the PSD. The GAP function of synGAP controls the activity of the GTPases Ras and Rap and thus regulates the Ras MEK-ERK pathway, which is involved in the insertion of AMPARs at the synapse. Over the last decade synGAP has been implicated in neurodevelopment disorders like autism spectrum disorders (ASD), intellectual disability (ID) and epilepsy. To understand why loss of synGAP causes an increase of AMPARs at the surface and thus impacts synaptic function we use super-resolution microscopy (SRM). Dual-color direct STochastic Optical Reconstruction Microscopy (d-STORM) enables us to observe alterations in receptor nano-organization as well as co-organization between different synaptic proteins e.g. synGAP, PSD-95 and AMPARs. To investigate changes in AMPAR nano-organization at different stages of development we used primary hippocampal neurons from a heterozygous synGAP mouse line and compared them to neurons from wild type littermates. In addition to the SRM approach we performed electrophysiological experiments measuring miniature excitatory post-synaptic currents (mEPSCs) and observed that reduced synGAP levels cause an increased mEPSC amplitude during development. Our goal is to understand the molecular mechanisms underlying changes in synaptic function which are caused by altered synGAP levels in developing neurons.

B35 - High resolution lattice light-sheet microscopy deeper in brain slices

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Lattice light-sheet microscopy (LLSM) stands out as the most successful and performant imaging technique to study 3D sub-cellular dynamic mechanisms in live thin samples[1]. In order to maintain a very high imaging quality in optically thick samples, Liu *et al.* developed an adaptive optics (AO)-LLSM[2] that corrects for sample induced optical aberrations occurring on the illumination and imaging paths. They were able to acquire fast volumetric events at very high spatial resolution within large samples, but to the cost of a complex and expensive instrument.

Here we propose an alternative, simpler, and cost-effective method to maintain sub-micrometric resolution deep into thick samples. We added a deformable mirror (DM) to the detection path of our LLSM[3,4] and developed a two-step active image optimization procedure: (1) light-sheet autofocus (AF), and (2) optimization of the DM shape to correct wavefront distortion (AO). Both AF and AO steps are based on an indirect, sensorless image-based process. We propose a fast and efficient AO optimization algorithm that minimizes the number of required images to provide an accurate correction. Several image metrics and iterative process were tested on a wide variety of samples. The optimized AO process provides a good robustness to photobleaching.

To test our AO technique, we artificially induced known aberrations on various non-aberrating samples (beads, HEK cells, neurons at the surface of brain slices). Our aberration correction method recovered a wavefront flatness of < 50 nm RMS, typically within < 40 s. In thicker samples, we demonstrated that both AF and AO steps are required to significantly increase brightness and resolution as shown in brain slices and in Arabidopsis root.

Even though our technique does not correct aberrations on the excitation path, we can still significantly improve LLSM image quality and thus extend its range of applications.

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