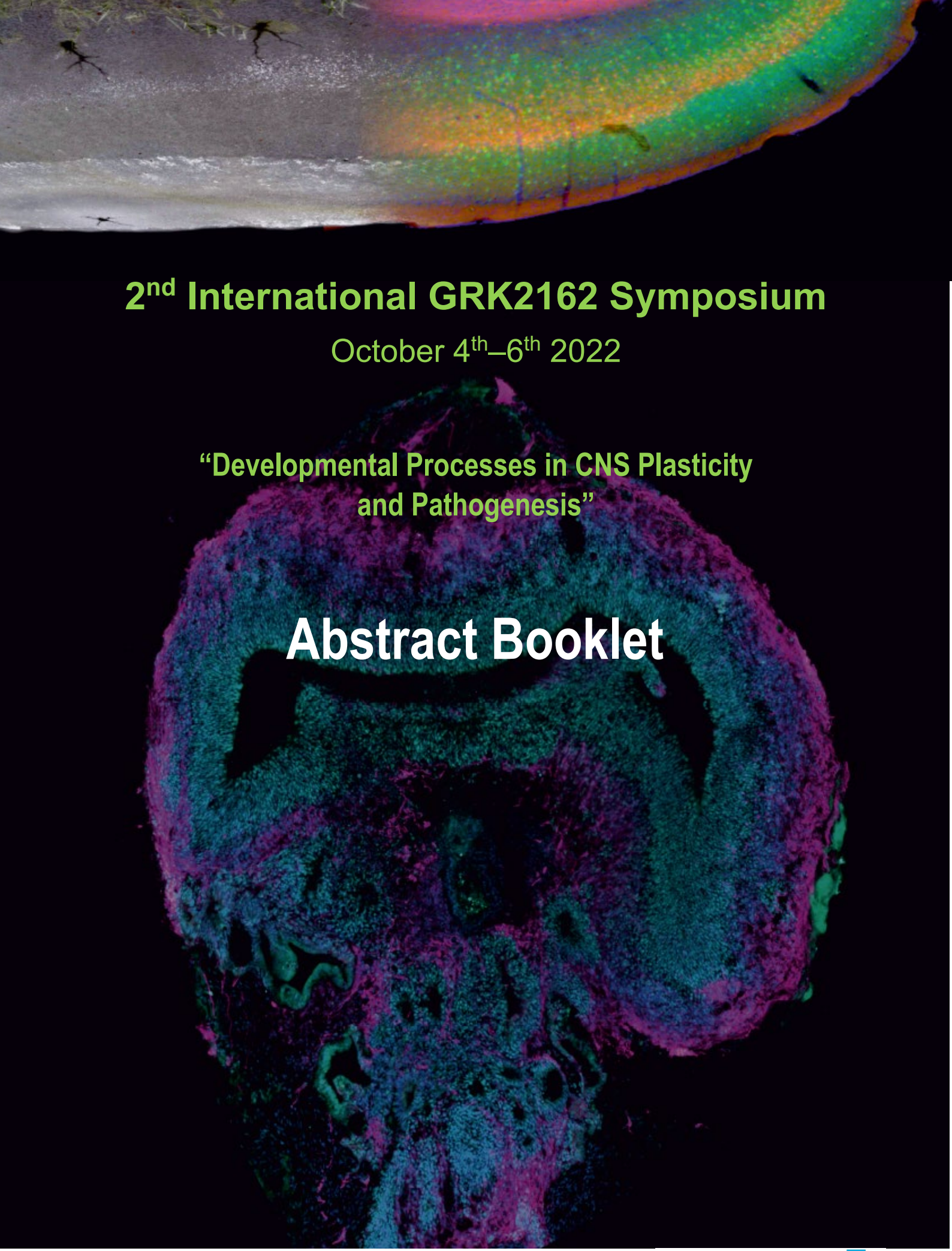

2nd International GRK2162 Symposium

October 4th–6th 2022

"Developmental Processes in CNS Plasticity and Pathogenesis"

Abstract Booklet

FAU Friedrich-Alexander-Universität Erlangen-Nürnberg

DFG-GRK2162 – "Neurodevelopment and Vulnerability of the Central Nervous System"

Universitätsklinikum Erlangen

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Abstracts and Titles of Speakers

A novel role of SOXC factors in proliferation and survival of adult neural stem and progenitor cells

Sneha Adhikarla[1], Benjamin Häberle[1], Iris Schäffner[1], Elizabeth Sock[1] and Dieter Chichung Lie[1].

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The dentate gyrus of the adult hippocampus is a dynamic structure in which new neurons and synaptic connections are constantly formed throughout life, even into adulthood. This process of adult neurogenesis is essential for neuronal plasticity, mood regulation and memory formation. The precise mechanisms underlying this process are still not fully understood. The SOXC family of transcription factors have been implicated in the neuronal specification of adult neural stem / precursor cells (ANSCs). Here, we uncover a new role for these transcription factors in proliferation and survival of ANSCs. Deletion of SOXC factors from ANSCs caused a loss of neurogenesis and death of intermediate precursor cells. Interestingly, in vitro analysis revealed the defects in mitosis and the formation of multinucleated cells in the absence of SOXC factors. Currently, we are working to determine how lack of SOXC factors induce this mitosis catastrophe and subsequent cell death. This study will elucidate the role of SOXC factors in the regulation of progenitor proliferation and survival, and help us extending our understanding of the process of adult neurogenesis.

The Development of an Adult Neurogenic Niche

Daniel Berg

University of Aberdeen, Institute of Medical Sciences, School of Medicine, Medical Sciences & Nutrition, Scotland, UK

The dentate gyrus of the hippocampus is different to most regions of the brain in that new neurons are continuously added throughout life in a process called adult neurogenesis. These new neurons are generated by radial glial-like neural stem cells that progress through brain development while retaining their stem cell potential. The cellular and molecular mechanisms that distinguish these neural stem cells from other developmental stem cells that deplete after completed development is not known. We developed a clonal analysis method that allows us to lineage trace the cells fated to become adult neural stem cells and to analyze their molecular signatures and cellular behavior. Using this technique, we were able to identify the embryonic origin of the adult neural stem cells and examine them at different stages of development. Moving forward we are attempting to identify the molecular determinants of neural stem cells fate in the developing dentate gyrus and analyzing how pathological perturbations affect this process.

Activating ß-glucocerebrosidase by exploiting its transporter LIMP-2

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Mutations in the GBA gene cause of the lysosomal storage disorder Gaucher disease and are among the highest genetic risk factors for the development of the neurodegenerative disorder, Parkinson disease (PD). GBA encodes the lysosomal enzyme beta-glucocerebrosidase (GCase), which orchestrates degradation of glucosylceramide (GluCer) in the lysosome. Impaired GluCer metabolism is associated with neuronal damage and neurodevelopmental defects. Recent studies have shown that GluCer accelerates alpha-synuclein aggregation, exposing GCase as a promising target in the treatment of PD. Ongoing research and clinical trials are aiming to identify GCase-activating compounds for clinical application.

In this study, we investigated the interaction of the lysosomal membrane protein and GCase transporter LIMP-2 with wild type (wt) GCase and the most prominent disease-associated GCase variants: E326K, N370S and L444P. Our data reveal that LIMP-2 not only mediates transport of GCase to the lysosome, but functions as a potent allosteric activator of the enzyme. Overexpression of LIMP-2 boosted lysosomal transport of GCase and rescued activity of the E326K variant in HEK293T cells. We found that a single helix in the LIMP-2 ectodomain was sufficient for activation of GCase in vitro. Based on this helix, we designed a custom lysosome-targeted peptide to restore lysosomal GCase activity in fibroblasts of PD patients harboring GBA E326K mutations. These findings expose the LIMP-2 interaction site on GCase as a potential therapeutic target to rescue lysosomal GCase activity, thereby revealing a novel approach for the design of GCase-activating compounds.

Possible role of NKCC1 in the fate of hippocampal neural stem cells during Alzheimer's disease

Anna-Lena Fleischer

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Alzheimer´s disease (AD) is one of the main neurodegenerative disorders of the 21st century, characterized by a progressive decline in cognitive function as manifested by a difficulty in learning new information and memory loss. Currently, AD is considered incurable and barely treatable, leading to an increasing need for patient care, posing enormous social and economic challenges to society and healthcare systems worldwide.

One important mechanism in memory formation and learning is adult neurogenesis, the process by which new neurons arise from neural stem cells in the dentate gyrus of the adult hippocampus. Current research of our group suggests that neural stem cells are affected in their fate decisions by AD. We observed an increased proliferation of neural stem- and progenitor cells in the pre-plaque phase which is followed by a strong decline in the number of stem cells and adult neurogenesis, and an increased astrogenesis. Factors modulating neural stem cell proliferation during adulthood and ageing are not well understood, however, our own preliminary data suggest that the loss of the co-transporter NKCC1 in stem cells alters proliferation behavior. The cation-chloride co-transporters NKCC1 and KCC2 are important modulators of intracellular chloride homeostasis and thus of the GABAergic system. The aim of the present study was to investigate the underlying mechanisms of early-onset of stem cell proliferation in AD. To this end, we quantified the expression levels of NKCC1 and KCC2 in hippocampal neural stem cells in young and middle-aged Nestin-GFP mice and APPxPS1/Nestin-GFP triple-transgenic mice, using confocal microscopy and 3D reconstruction with Imaris software. During ageing, levels of the NKCC1 co-transporter significantly decreased in the Nestin-positive stem cells. Importantly, this age-related decrease was already seen in the Alzheimer's animals in the pre-plaque phase. On the contrary, in the aged stem cells the KCC2 co-transporter was increased. Our results suggest that alteration of NKCC1 and KCC2 play an important role in the early overactivation of adult neural stem cells during Alzheimer's disease.

Analysis of ectopic Sox9 expression during different timepoints of oligodendrocyte development in the murine CNS

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Repair processes in the diseased or injured adult brain are often dependent on the reactivation of processes that are usually active during ontogenetic development. In the case of demyelinating diseases such as Multiple Sclerosis, repair requires the generation of new oligodendrocytes and myelin by NG2 glia. Characteristically, early postnatal NG2 glia proliferate and differentiate quite efficiently as well as express Sox9 endogenously. On the other hand, adult NG2 glia do not maintain this efficient differentiation ability and, interestingly, do not express Sox9. Therefore, we investigate whether this difference in Sox9 expression could be the key to more easily differentiating adult NG2 glia. We show that the overexpression of Sox9 in developing and early postnatal NG2 glia leads to an increase in their ability to proliferate and differentiate. We also show that ectopic Sox9 expression in adult NG2 glia causes these cells to differentiate more efficiently and effectively. Sox9 could be one of the key regulators in NG2 glia differentiation, opening the door to future therapies in demyelinating diseases.

PRC2-mediated repression is essential to maintain identity and function of differentiated dopaminergic and serotonergic neurons

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The brain contains a large number of different neuronal subtypes that maintain their distinct cellular identities over several decades despite continuous environmental fluctuation. Apart from the instructive information provided by transcription factors controlling cell type-specific gene programs, there is also a need to stably maintain silencing of transcriptional programs governing other cellular fates. The mechanisms regulating such enduring silencing in mature neurons are not well understood. Our work addresses the role of the Polycomb repressive complex 2 (PRC2) in the maintenance of identity in differentiated dopaminergic and serotonergic neurons. Deletion of the obligate PRC2-component, Eed, in these neurons, resulted in progressive global loss of H3K27me3, followed by a gradual activation of genes harbouring both H3K27me3 and H3K9me3 modifications. Notably, H3K9me3 was lost at these PRC2-targets prior to gene activation. Neuronal survival was not compromised, instead there was a reduction in subtype specific gene expression and a progressive impairment of dopaminergic and serotonergic neuronal function leading to behavioural deficits characteristic of Parkinson's disease and anxiety. Single cell analysis revealed subtype-specific vulnerability to loss of PRC2 repression in dopamine neurons of the substantia nigra. Taken together, our results reveal that a PRC2-dependent non-permissive chromatin state is essential to maintain subtype identity and function of dopaminergic and serotonergic neurons.

Learning from Schwann cells to promote oligodendrocyte differentiation and plasticity after lesion

Claire Jacob

Johannes Gutenberg University Mainz

Abstract : The work of my lab aims at elucidating mechanisms that can be used to promote the regeneration of the nervous system after a lesion due to a traumatic injury such as a spinal cord injury or to a demyelinating disease such as multiple sclerosis. In particular, we are very interested in understanding how neuron/glia interactions and chromatin-remodeling enzymes can modulate the regeneration capacity of the peripheral and central nervous systems (PNS and CNS, respectively). The PNS has a high capacity of regeneration after lesion, whereas regeneration of the CNS is very limited. This is in part due to the high plasticity of Schwann cells, the myelinating glia of the PNS. One of our approaches to promote CNS regeneration is to identify mechanisms controlling the plasticity and differentiation of Schwann cells, and to transfer or use these mechanisms in oligodendrocytes to increase their plasticity and differentiation after lesion and thereby promote CNS regeneration. During the symposium, I will present our recent findings on this topic.

Molecular and functional heterogeneity of neural stem cells

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Neural stem cells generate new neurons throughout life in distinct regions of the mammalian brain. This process, called adult neurogenesis, is critically involved in certain forms of learning and memory. In addition, failing or altered neurogenesis has been associated with a number of neuro-psychiatric diseases such as major depression and cognitive aging. We aim to characterize the cellular and molecular mechanisms regulating neural stem cell activity and behavior on a single cell level. We present new approaches to study the cellular principles underlying life-long neurogenesis using imaging-based tools and single cell molecular profiling. Further, we provide evidence for novel molecular mechanisms governing the neurogenic process in the mammalian brain. Thus, the data presented provide new insights into the cellular principles of hippocampal neurogenesis and identify novel mechanisms regulating the behavior of rodent and human neural stem cells.

CRISPR-based dissection of inflammatory CNS lesion formation

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The common neuroinflammatory condition multiple sclerosis (MS) is initiated when activated autoreactice T cells enter the central nervous system (CNS) where they trigger the formation of focal inflammatory lesions characterized by demyelination and axon loss. If we want to treat MS efficiently, we thus need to understand the key molecules that regulate the immunopathogenesis of inflammatory lesion formation in the CNS. In the recent years, we have now established CRISPR-based screening approaches that allow us to comprehensively characterize the molecular regulation of inflammatory CNS lesion formation in preclinical MS models. Here I will introduce these approaches that target i) the infiltration of autoreactive CD4+ T cells into the CNS, the step that initiates lesion formation in the first place and ii) the local proinflammatory activation of monocyte-derived macrophages that is critical for CNS tissue damage. I hope to illustrate in my presentation how the unbiased molecular dissection of critical steps in MS pathogenesis enabled by CRISPR-based screens can help us describe the essential endogenous facilitators and brakes of inflammatory lesion formation, identify the functional modules to which they belong and delineate targets for therapeutic intervention.

Tissue mechanics-modulating small leucine-rich proteoglycans contribute to the differential regenerative capacity of mammalian and zebrafish central nervous system axons

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Spinal cord injury results in the formation of fibrous scar tissue in mammals. The scar forms through deposition of extracellular matrix (ECM) and constitutes a hostile environment to axon regeneration, thereby preventing recovery of function. In contrast, the ECM in the spinal lesion site of zebrafish is permissive and required for regeneration. Whether interspecies differences exist in the composition of the injury ECM that contributes to regeneration failure and success has, however, not been systematically addressed.

We performed cross-species comparative proteomics to identify differences in the ECM composition of spinal cord-lesioned zebrafish and rat. Differentially enriched candidate proteins were analyzed for axon growth-modulating properties, using a range of in vivo and in vitro assays.

We identified members of the small leucine-rich proteoglycan (SLRP) family to exhibit high abundance in rat and human central nervous system (CNS) lesions but not in regenerative-competent zebrafish. Experimentally increasing the protein levels of individual SLRPs in the zebrafish lesion environment inhibits axon regeneration and functional recovery. Mechanistically, we obtained evidence that SLRPs modulate the mechanical properties of the lesion environment toward a non-permissive signature.

Our data identified SLRPs as previously unrecognized axon growth-limiting ECM components that contribute to the differential regenerative capacity of mammalian and zebrafish CNS axons, and indicate an impact of tissue mechanics on the regeneration outcome.

Autophagy regulates neuronal excitability by controlling cAMP/Protein Kinase A signaling

Natalia Kononenko

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Autophagy provides nutrients during starvation and eliminates detrimental cellular components. However, accumulating evidence indicates that autophagy is not merely a housekeeping process. Here, by using neuronal-confined mouse models of ATG5 deficiency in either excitatory or inhibitory neurons and a combination of SILAC and label-free quantitative proteomics, high-content microscopy, and live-imaging approaches, we show that the protein AuTophaGy 5 (ATG5) functions in neurons to regulate the cAMP-dependent protein kinase A (PKA)-mediated phosphorylation of a synapse-confined proteome. This function of ATG5 is independent of bulk turnover of synaptic proteins and requires the targeting of PKA inhibitory R1 subunits to autophagosomes. Neuronal loss of ATG5 causes synaptic accumulation of PKA R1, which sequesters the PKA catalytic subunit and diminishes the cAMP/PKA-dependent phosphorylation of postsynaptic cytoskeletal proteins mediating AMPAR trafficking. Glutamatergic neurons-confined ATG5 deletion augments AMPAR-dependent excitatory neurotransmission and causes the appearance of spontaneous recurrent seizures in mice. Our findings identify a novel role of autophagy in regulating PKA signaling at glutamatergic synapses and suggest the PKA as a target for restoration of synaptic function in neurodegenerative conditions with autophagy dysfunction.

Involvement of microglia in Hereditary Spastic Paraplegia type 11

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Neuroinflammation, and microglia, in particular, have emerged as key players in the pathogenesis of neurodegenerative and motor neuron diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). In a recently published mouse model of another rare motor neuron disorder, Hereditary Spastic Paraplegia type 11 (SPG11-HSP), pathogenic neuroinflammation including microgliosis was also found in distinct compartments of the diseased CNS. SPG11-HSP is a complex form of HSP that is caused by pathogenic variants in the SPG11 gene. The disease is characterized by progressive weakness and spasticity of the lower limbs besides additional neurological symptoms such as thinning of the corpus callosum and cortical atrophy. Here, we demonstrate for the first time an inflammatory pathology in a human model of SPG11-HSP. Analyzing the serum of SPG11-HSP patients, elevated levels of proinflammatory resistin and reduced levels of the important immunomodulator TGFβ1 were revealed in comparison to healthy controls. To investigate if microglia that lack SPG11 encoded spatacsin are affected in a cell-autonomous way, we aimed to functionally characterize iPSC-derived microglia-like cells (iMGLs) from three SPG11-HSP patients (SPG11-iMGL). SPG11-iPSC differentiated equally into iMGL in comparison to control iMGL and did not feature any lipid or lysosomal accumulations. However, all SPG11-iMGLs did produce and secrete more proinflammatory cytokines like IL-1β and TNFα specifically upon IFNγ treatment, indicating an over-active microglial stage. Although the exact correlations still need to be determined, we propose that neuroinflammation is contributing to SPG11-HSP pathology and that microglia are affected in a cell-autonomous way. To further validate our hypothesis, postmortem brain tissue from SPG11-HSP patients will additionally be examined for microglia-associated pathology. Clarifying the precise role of the immune system in SPG11-HSP brings us closer to uncovering the pathological mechanism and finding therapeutic approaches.

Exciting complexity: Circuit mechanisms of cortical hyperexcitability in ALS

Sabine Liebscher

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Amyotrophic lateral sclerosis (ALS) is fatal neurodegenerative disease primarily characterized by the degeneration of upper (UMN) and lower motor neurons (LMN) in the cortex and the spinal cord, respectively. Despite an ever-growing list of molecular targets this insidious disease is still incurable leading to death within only a handful of years upon diagnosis.

Research in ALS has strongly focused on unravelling the molecular mechanisms leading to lower motor neuron loss, while little is known about the mechanisms triggering upper motor neuron degeneration. Notably, cortical hyperexcitability is a key feature of sporadic and familial ALS forms, which precedes overt motor symptom onset, and which is sufficient to trigger UMN and LMN degeneration. We thus here set out to identify the circuit mechanisms underlying cortical hyperexcitability in ALS. To this end, we performed in vivo two-photon calcium imaging in behaving ALS transgenic mice ($SOD1^{G93A}$) using a visual-flow feedback paradigm. Unexpectantly, we identified a hitherto unrecognised non-cell autonomous mechanism, causing excessive glutamatergic stimulation of UMNs. Chemogenetic manipulation of the identified neuronal population slows down disease onset and progression. Thus, our data argue for a circuit mechanism underlying cortical hyperexcitability in ALS and offer a novel therapeutic entry point.

Mechanisms of Voltage-Gated Potassium Channel distribution in myelinated axons

Dies Meijer

University of Edinburgh, United Kingdom

The role of Kv1 channel complexes at the juxtaparanodal membrane of myelinated axons has remained controversial. Two questions will be addressed: First, what mechanisms drive the clustering of the Kv1 channels at the juxtaparanode, and second, what role do these Kv1 channels play in the physiology of the myelinated axon.

The generally accepted view is that Kv1 channels are recruited and stabilized at the juxtaparanodal membrane during postnatal development by the cell adhesion molecules CASPR2 and TAG-1 and subsequently link the complexes to the underlying actin/spectrin network. We challenge this view and demonstrate that Kv1 channels are recruited to the JXP membrane by the cell surface receptor ADAM23 and is soluble ligands LGI3 and LGI2. We further demonstrate that juxtaparanodal Kv1 channels contribute to the re-charging of the nodal membrane following an action potential.

Harnessing intrinsic properties for engineering human brain-region specific organoids using induced pluripotent stem cells

Guo-li Ming

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Human Induced pluripotent stem cells (hiPSCs) have the intrinsic potential to generate all cell types of a human body under 2D culture conditions. hiPSCs can also be directed to form organ like structures-organoids under 3D culture conditions, including brain organoids resembling the developing brain. Human brain organoids offer unique advantages in understanding molecular and cellular mechanisms governing embryonic neural development and in modeling neurodevelopmental disorders. I will discuss our recent work in developing protocols for generating brain-region specific organoids by first specifying hiPSCs into brain region specific neural stem cells, which are capable of self-organizing into distinct brain structures. I will also discuss our work on using these organoid models to understand molecular and cellular mechanisms underlying neurodevelopmental disorders.

Autophagy is a cell-intrinsic driver of neural stem cell quiescence in hippocampal dentate gyrus development

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Neurogenesis in the adult mammalian brain relies on the life-long persistence of quiescent neural stem cell (NSC) reservoirs. Little is known about the mechanisms that lead to the initial establishment of NSC quiescence during development. We here show that the autophagy machinery accumulates in quiescent NSCs and that pharmacological blockade of autophagy disrupts quiescence. We further demonstrate that autophagy is cell-intrinsically required to establish radial glia-like NSC quiescence during hippocampal development.

Brain cell-type regulatory landscapes and associations with disease

Alexander Nott

Group Leader and UK DRI Fellow, UK Dementia Research Institute at Imperial College London, Department of Brain Sciences, LGBTQ Champion

Genetic variants have been identified that increase the risk of aging-associated brain disorders such as Parkinson's disease and Alzheimer's diseases (AD), however, the function of these variants is largely unknown. They often reside in noncoding regions thought to regulate gene expression, called enhancers. Enhancers integrate environmental signals, resulting in gene expression programs that guide cell type-specific responses. The localization of disease-risk variants in enhancers suggest that enhancer function is critical for normal aging and disease. My research is to decipher how cell type-specific enhancer function drives brain physiology and aging-related disease. To address this problem, we established a nuclei isolation protocol for specific cell types of the human brain. Utilizing this approach, we generated enhancer-promoter interactome maps for brain cell types of the human brain from non-dementia individuals. This epigenomic atlas has identified the gene repertoire influenced by disease-risk variants and revealed the probable cell types in which they function. To capture disease-relevant regions in the context of aging-related brain diseases, we are mapping cell-type enhancer landscapes in the AD brain. Collectively, these studies will reveal cell type-specific gene networks and transcription factors important for brain aging and highlight candidate targets that are dysregulated in disease.

The rapid developmental rise of somatic inhibition disengages hippocampal dynamics from self-motion

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Early electrophysiological brain oscillations recorded in preterm babies and newborn rodents are initially mostly ignited by bottom-up sensorimotor activity and only later can detach from external inputs. This is a hallmark of most developing brain areas including the hippocampus, which in the adult brain, functions in integrating external inputs onto internal dynamics. Such developmental disengagement from external inputs is likely a fundamental step for the proper development of cognitive internal models. Despite its importance, the developmental timeline and circuit basis for this disengagement remain unknown. To address this issue, we have investigated the daily evolution of CA1 dynamics and underlying circuits during the first two postnatal weeks of mouse development using two-photon calcium imaging in non-anesthetized pups. We show that the first postnatal week ends with an abrupt shift in the representation of self-motion in CA1. Indeed, most CA1 pyramidal cells switch from activated to inhibited by self-generated movements at the end of the first postnatal week whereas the majority of GABAergic neurons remain positively modulated throughout this period. This rapid switch occurs within two days and follows the rapid anatomical and functional surge of local somatic GABAergic innervation.

Myelin in motor learning, cognition and memory

William D. Richardson

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Generation of new myelinating oligodendrocytes (OLs) from their precursor cells is stimulated by – and required for – motor skill learning in mice. Involvement of myelin in learning and memory is also suggested by MRI studies that reveal microstructural changes in white matter tracts of people who learn a new visuomotor skill, or who engage in a cognitive task such as learning a second language or training to improve working memory. We asked whether new OL production is required for training-dependent memory improvement in mice, using T-maze and 8-arm radial maze tasks designed to exercise and evaluate spatial working memory. Blocking OL genesis by conditional knockout of the transcription factor Myrf in OL precursors impaired the ability of adult mice to improve their performance in these tasks over 8 days of training, relative to control littermates. In wild type mice, maze training stimulated production of additional OLs and myelin in the anterior cingulate cortex and underlying corpus callosum – regions known to be involved in working memory processes. Strikingly, there was a close correlation (R^2>0.7) between the number of new OLs generated in those brain regions during training and ultimate performance score in the radial maze. These results suggest an essential and rather direct requirement for OLs and myelin in working memory performance, which is known to underpin all kinds of cognitive abilities and correlates strongly with measures of “fluid intelligence” in humans. Since both motor skill learning and working memory improvement depend on practice over days, we propose that, in general, OL generation is required for learning and memory processes that depend on reiterative training.

P97/VCP and autophagy

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Intracellular protein aggregation is a feature of many late-onset neurodegenerative diseases, including Parkinson's disease, tauopathies, and polyglutamine expansion diseases (like Huntington's disease (HD)). Many of these mutant proteins, like that causing HD, cause disease via toxic gain-of-function mechanisms. Therefore, the factors regulating their clearance are crucial for understanding disease pathogenesis and for developing rational therapeutic strategies.

We showed that autophagy induction reduces the levels of mutant huntingtin and attenuated its toxicity in cells, and in *Drosophila, zebrafish* and mouse HD models. We have extended the range of intracellular proteinopathy substrates that are cleared by autophagy to other related neuro-degenerative disease targets, like alpha-synuclein in Parkinson's disease and tau in various dementias and Alzheimer's disease. I will describe how autophagy is compromised in certain neurodegenerative diseases, focussing on VCP/p97, where loss-of-function can cause tauopathy and dementia. I will then consider how autophagy induction may be a powerful therapeutic approach for some of these conditions.

Impact of activin signalling on hippocampal GABAergic inhibition and its effects in neuropsychiatric disorders

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Adolescence is a critical period associated with behavioural and emotional changes. Aversive challenges like chronic stress during this period may increase susceptibility to neuropsychiatric disorders later in life. However, the pathophysiological mechanisms rendering the adult brain susceptible remain largely unknown. Studies have indicated an increase in GABAA receptors during this late developmental stage. Our previous work showed that activin, a member of the TGF-β family, modulates hippocampal GABAergic inhibition and impacts anxiety-like behaviour, using transgenic mice expressing dominant-negative activin receptor IB (dnActRIB). To compare the neuronal circuitry during adolescence (P30-45) and in adulthood (P90-P120), we first systemically examined GABAergic inhibition onto granule cells in dentate gyrus, a hippocampal sub-region closely linked to the antidepressant effect of activin signalling. Whole-cell voltage-clamp recordings from hippocampal slices showed a significant increase in GABAergic inhibitory postsynaptic currents (IPSCs) in granule cells from adolescent mice, compared to that from adult slices. Interestingly, acutely applied corticosterone produced mixed responses of IPSCs in adult dorsal slices, but a uniform suppression in ventral ones, indicating diverse impacts of stress hormone along hippocampal longitudinal axis. To elucidate the mechanisms involved in adolescent stress and its effects on adulthood, we then established a behaviour model by administering corticosterone during adolescence (P30-P45). Depression-like phenotype in adulthood was manifested in forced swim test with higher immobility in treated wild type, but not dnActRIB, mice. Preliminary data from hippocampal granule cells from stressed mice indicate a tendency of decrease in IPSC frequency, suggesting the long-lasting impact of adolescent stress on hippocampal synaptic transmission.

Human dorsal forebrain organoids help to elucidate cell type-specific effects of maternal immune activation on neocortical development

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Prenatal exposure to maternal immune activation (MIA) during the first trimester of gestation is correlated with long-term deficits in brain development in the offspring, including increased risks for autism spectrum disorder (ASD) in humans. Rodent models show causal links between prenatal exposure to MIA and ASD-like behavioral deficits in pups that are mediated by deficits in protein translation in neurons. While the majority of studies on the effects of MIA focus on neuronal abnormalities, a growing body of evidence suggests that defects in proliferative and neurogenic capacity of neural stem cells may be important contributors to ASD. In order to determine changes in neurogenesis following MIA in human neural stem cells, we treat human iPSC-derived dorsal forebrain organoids with molecular mediators of MIA. We have validated our model by showing that both dorsal forebrain organoids and midgestational human neocortex express the molecular machinery necessary for the response to the mediators of MIA. We have investigated the consequences of signaling pathway activation by mediators of MIA using immunohistochemistry and Western Blotting. Single-cell RNA sequencing of more than 10,000 cells showed that dorsal forebrain organoids represent the diversity of cell types in the developing human neocortex. While mediators of MIA do not significantly affect cellular composition of organoids, they induce differential gene expression in them. Currently, we are characterizing differentially expressed genes in distinct cell types. Taken together, we have established a human *in vitro* model system of MIA that allows us to untangle cellular and molecular changes at an unprecedented resolution.

Modeling Neuro-Immune Interactions under Physiological Conditions: A novel Organoid-based Approach for Studying Human Environment-dependent Microglia Phenotypes

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Microglia are a specialized population of brain-resident macrophages that play a central role in brain development. However, until now, the ability to model the interactions between the human brain environment and microglia has been severely limited. To overcome these limitations, we have developed a novel approach that leverages recent advances in stem cell biology to mimic the invasion of erythromyeloid progenitors into cortical brain organoids and capitalizes on our ability to graft these units into a rodent host for vascularization. The integrated microglial cells (hMG) survive for extended periods of time, express microglia-specific markers and populate the human organoid graft. Furthermore, hMG show morphological features indicative of a resting and surveillance state and assume transcriptomic signatures that closely resemble their in vivo counterparts. Taken together, the system developed here will provide a platform for studying functional human brain-microglia interactions under physiological conditions and over extended periods of time, a critical next step for modeling human brain environment-dependent microglia phenotypes in health and disease.

Lysosomal acidification and function is modulated by FoxO-signaling in adult hippocampal neural/stem progenitor cells

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Ensuring adult neural stem cell function in the mammalian brain over time is controlled by niche derived factors and stem cell's endogenous transcriptional programs. Furthermore, studies from the last decade have shown that the metabolic system and the proteasome and autophagy-lysosome pathway (ALP) system play a crucial role in adult neural stem cell homeostasis and maintenance of adult neurogenesis. The mechanisms controlling ALP activity in adult neural stem cells and subsequently generated neurons are largely unknown. We recently identified transcription factors of the FoxO family as central regulators of ALP activity in adult hippocampal neurogenesis with ramifications for neural stem cell homeostasis and for morphology of newborn neurons. Our new findings in FoxO-deficient adult hippocampal neural stem/progenitor cells indicate insufficient acidification of lysosomes, which in turn leads to the accumulation of fused autolysosomes and the blockage of the following degradation process. The lysosomal pH is regulated by the vacuolar ATPase (v-ATPase), a multi-subunit proton pump located in the lysosomal membrane, which pumps protons into the lysosomal lumen under ATP hydrolysis upon its reversable assembly. We identified subunits of the v-ATPase as well as modulators of both v-ATPase function and lysosomal enzymes as potential targets of FoxO signaling.
Our recent findings suggest a new link between FoxO transcription factors and v-ATPase-dependent lysosomal acidification and function in adult neural stem/progenitor cells.

Continuous neurogenesis in the mammalian hippocampus across the lifespan

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Immature dentate granule cells arising from adult hippocampal neurogenesis contribute to plasticity and unique brain functions in rodents and they are dysregulated in many human neurological disorders. Using genetic clonal lineage-tracing, we identified a pool of neural stem cells that exhibit similar molecular and epigenetic characteristics and continuously generate dentate granule neurons throughout life in the mouse hippocampus. Single-cell RNA-seq analysis further reveals a transition from active to quiescent neural stem cell state during the early postnatal stage followed by a continuous maturation process. Rather than categorize hippocampal neurogenesis into two stages of "embryonic neurogenesis" vs. "adult neurogenesis", we propose a continuous model of neurogenesis suggesting that dentate gyrus development is a life-long process. Using single-nucleus RNA-seq of postmortem human samples, we further provide evidence for the present of immature dentate granule neurons and identify their molecular characteristics in the human hippocampus across the lifespan and in patients with Alzheimer's disease.

Studying the impact of interferon-gamma (IFN-γ) on development and relevance for neurodevelopmental disorders

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Multiple lines of evidence indicates that maternal immune activation (MIA) is linked with increased likelihood of a neurodevelopmental and psychiatric disorders. The origins and molecular mechanisms that underlie the increased chances for these disorders are thought to be driven by activation of the immune system which subsequently influences neurodevelopment. It is thought that inflammatory cytokines represent one critical link between prenatal immune activation and long-lasting changes in brain development, ultimately resulting in the emergence of behavioural phenotypes relevant for neurodevelopmental conditions and psychiatric disorders.

A number of canonical pro-inflammatory cytokines have been linked with MIA – including members of the interleukin family of cytokines. However, elevated levels of interferon-gamma (IFN-ɣ) have been observed in both human and animal studies of MIA. IFN-ɣ is an activator of innate cellular antiviral signalling and transcription programs whose primary function is to defend the cell against viral infection and in response to immune activation. Interestingly, it is also emerging that IFN-γ has a physiological role beyond its antiviral and immune actions. Indeed, IFN-γ and its signalling targets have been described to have play important roles in neuronal development and synaptic activity, independent of microbial infection. However, it is unclear how elevated levels of IFN-γ may contribute to increased likelihood of neurodevelopmental conditions and psychiatric disorders.

To this end, we have used human induced pluripotent stem cells (hiPSC) to model early neurodevelopment and to investigate whether IFN-γ can persistently influence the development of forebrain neurons and induce transcriptional changes in hiPSC neural cells relevant for neurodevelopmental conditions and psychiatric disorders. We have furthermore used hiPSC cells derived from indivuduals wioth schizophrenia or autism to investigate how a genetic background associate with these conditions may impact the effect of IFN-γ in developing human neurons. Our findings suggest that a transient exposure to IFN-γ persistently alters the development of hiPSC-forebrain neurons, induces a transcriptomic profile consistent with that seen in the brains of individuals with schizophrenia or autism, and that hiPSC-neural cells with a genetic background associated with these conditions alter the cellular response to IFN-γ.

Roles of long-lived cellular constituents in the maintenance of brain function

Tomohisa Toda

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Ageing is one of the most critical risk factors for neurological and psychiatric diseases. However, the biological links between physiological ageing and pathological development are still largely unknown. A solid understanding of the biology of brain ageing will thus be a key to developing the means to treat these diseases. Since neurons and adult neural progenitor cells in the brain are mostly generated during development with limited capacity of replacement, they need to maintain their identity and function throughout our lives. We recently discovered that a cell type-specific nuclear architecture organized by nucleoporins and nuclear lamins regulates the maintenance of neural progenitor cells (NPs). Strikingly, nucleoporins and lamins are the most long-lived proteins in a cell and are known to be damaged during brain ageing, but their functional contribution in brain function and aging remain largely elusive. To understand how nuclear structural proteins regulate cell type-specific epigenetic programs, we combined interdisciplinary approaches including biochemical and epigenetic profiling. Our data indicate that nucleoporins interact with several chromatin regulators and work as a structural gatekeeper for cell type-specific gene expression. These data highlight novel roles of nuclear structural proteins in epigenetic regulation, and provide possible links between neural aging and brain dysfunction.

Tumor necrosis factor alpha (TNFα) modulates synaptopodin-dependent synaptic plasticity

Andreas Vlachos

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Microglia are the resident immune cells of the central nervous system (CNS). They play fundamental roles in active immune defense and neuroinflammatory responses. Historically, it has been assumed that microglia exist in a resting state until pathological stimuli trigger their activation. However, a series of recent landmark studies revealed important physiological functions of microglia in neural development, synaptic remodeling and homeostasis. Likewise, accumulating evidence suggests that immune mediators and inflammatory cytokines may assert physiological functions in synaptic transmission and plasticity. However, the neuronal targets and mechanisms through which microglia assert their effects on synaptic plasticity remain not well understood. My presentation focuses on the effects of microglial TNFα on synaptic plasticity and the role of synaptopodin-associated intracellular calcium stores. I will present unpublished work in which we aim at translating these findings to the human cortex toward a better understanding of how microglia modulate synaptic plasticity in health and disease.

Poster Session 1 – Wednesday Oct. 5th , 12:30 – 2:30 pm

A novel role of SOXC factors in proliferation and survival of adult neural stem and progenitor cells

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The dentate gyrus of the adult hippocampus is a dynamic structure in which new neurons and synaptic connections are constantly formed throughout life, even into adulthood. This process of adult neurogenesis is essential for neuronal plasticity, mood regulation and memory formation. The precise mechanisms underlying this process are still not fully understood. The SOXC family of transcription factors have been implicated in the neuronal specification of adult neural stem / precursor cells (ANSCs). Here, we uncover a new role for these transcription factors in proliferation and survival of ANSCs. Deletion of SOXC factors from ANSCs caused a loss of neurogenesis and death of intermediate precursor cells. Interestingly, in vitro analysis revealed the defects in mitosis and the formation of multinucleated cells in the absence of SOXC factors. Currently, we are working to determine how lack of SOXC factors induce this mitosis catastrophe and subsequent cell death. This study will elucidate the role of SOXC factors in the regulation of progenitor proliferation and survival, and help us extending our understanding of the process of adult neurogenesis.

Lysosome function in neural stem cell homeostasis in the adult and aged hippocampus

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Radial glia-like neural stem cells (NSCs) in the dentate gyrus (DG) of the hippocampal formation give rise to new neurons throughout life. The continuous generation of dentate granule neurons is associated with specific learning and memory processes. During aging, the rate of adult hippocampal neurogenesis declines sharply due to an exhaustion of the NSC pool. Age-associated reduction of adult hippocampal neurogenesis occurs as early as in 5-month-old mice, but underlying mechanisms remain largely ambiguous. Recent studies have shown that impaired lysosomal activity in NSCs is associated with deficits in hippocampal neurogenesis. Moreover, loss of function of TFEB (Transcription Factor EB), master regulator of lysosomal biogenesis, in NSCs causes increased activation of the NSC pool.
In this study, we investigate whether TFEB over-expression will elevate the activity of lysosomes and will be sufficient to balance neurogenesis and counter the depletion of the NSC pool during aging. To this end, we overexpressed TFEB in hippocampal NSCs of mice starting from the young adult stage. First analyses revealed that short-term (three days) and long-term (four months) overexpression of TFEB resulted in decreased NSC activation and proliferation and increased the abundance of quiescent NSCs in the DG. Exposure to exercise conditions revealed that quiescent NSCs could still be efficiently activated. Intriguingly, despite the decrease in NSC activation and proliferation, the overall number of newly generated mature neurons in the DG did not differ from control conditions.
These data suggest that augmenting TFEB function may promote long-term RGL NSC homeostasis and the generation of functional neurons in the aged brain. Future immunohistochemical, transcriptomic, and metabolomic analyses will shed light on the mechanisms underlying TFEB-mediated enhancement of adult NSC homeostasis in the aging mouse DG.

The role of Sox11 in cortical interneuron development

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SOX11 is a transcription factor with pleiotropic roles in embryonic and post-natal neurodevelopment, during which it regulates proliferation, cell fate determination, migration, and network integration. SOX11 pathogenic variants are associated with Coffin-Siris- like Syndrome (CSS9), a congenital defect resulting in microcephaly, delayed development, and intellectual disability.
We show high SOX11 expression in the mouse medial ganglionic eminence (MGE), the main neurogenic niche for the majority of cortical interneurons (IN) that are the main source of inhibition in the neocortex and are crucial for regulating information processing and cellular communication. The temporal overlap of SOX11 expression in the MGE during its peak neurogenic period suggests a role of SOX11 in murine cortical IN development. Indeed, preliminary results suggest an important role of SOX11 on IN development: $SOX11^{+/-}$ mice show an overall decrease parvalbumin (PV^{+}) INs in the dentate gyrus of the hippocampus (DG), reduced learning capacity, and altered PV-derived electrophysiological activity in the dorsal DG.
To investigate if SOX11 regulates IN development in a cell-autonomous manner, we are currently performing a detailed analysis of the temporal and spatial expression of SOX11 in the IN lineage and are generating conditional knockout mouse lines to ablate Sox11 specifically from IN precursors during embryonic development.

The role of Sox9 in regulating the neuron/glial switch of adult hippocampal neural stem cells

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The dentate gyrus (DG) is a unique structure in the brain. Persisting adult neurogenesis by neural stem cells (NSCs) warrants an individual´s ability to adapt to a changing environment. Besides neurogenesis, niche astrocytes contribute to the high level of plasticity in the DG. Adult NSCs generate neurons and astrocytes. Interestingly, our group revealed that the balance between neuro- and astrogenesis remains constant in the adult hippocampus. This suggests a mechanism that regulates the fate decision of adult NSCs. Which factors are responsible in controlling the neuron-to-astrocyte ratio? The transcription factor Sox9 emerged as a master-regulator of the neuron/glial switch during development (Klum et al., 2018) and governs astroglial fate of subventricular zone NSCs (Cheng et al, 2009).

To reveal mechanisms controlling neurogenesis versus astrogenesis in the adult DG, we use genetic mouse models to delete (Sox9ko) and overexpress Sox9 (Sox9oe) in adult hippocampal NSCs, respectively. Here, I observed an increase in astrogenesis at the expense of neurogenesis upon Sox9oe. Surprisingly, the majority of NSC descendants proved to be “hybrid cells” (simultaneously expressing neuronal Dcx and astroglial S100β) upon Sox9oe. In line, conditional Sox9ko in adult NSCs reduced newborn astrocyte numbers while neuroblast numbers did not change. In summary, Sox9 is involved in the neuron/glial switch in adult NSCs of the DG. Next, we will reveal downstream targets of Sox9 mediating pro-astroglial effects in NSCs. This will significantly promote our understanding of adult NSC behavior, which is a prerequisite to better understand glio- and neuropathological phenotypes.

Contribution of astrocytes to synapse formation in newly generated neurons in the adult hippocampus

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We have recently identified that the adult mouse dentate gyrus (DG) harbors astrocyte subtypes, which localize to distinct DG compartments and display distinct diversity on a molecular, morphological and physiological level. Single cell RNA sequencing revealed that many genes are differentially regulated between astrocyte subtypes. Here, specifically Neurexin 1 (Nrxn1) caught our attention being the only differentially upregulated gene astrocytes located in the molecular layer. While we know neuronal Nrxn1 is pivotal to the formation and function of synapses, it is unclear what role astrocytic Nrxn1 may play in this same regard. Importantly, as one of two regions where adult neurogenesis occurs, the DG offers a unique opportunity to elucidate mechanisms behind maturation and integration of newly generated neurons in the adult brain, as well as overall circuit plasticity. The known role of neuronal Nrxn1 in synapse formation together with its differential upregulation in astrocytes, which are in direct contact with the dendrites of newborn neurons led us to speculate that. Understanding how these diverse astrocytes, and Nrxn1, interact with granule neurons in normal versus pathological states we improve our therapeutic strategies or targets moving forward. To address this I will use a genetic approach by conditionally knocking out (cKO) Nrxn1 in DG astrocytes in adult mice. Upon cKO of Nrxn1, I will examine the effects on neurogenesis through immunohistochemistry and birth dating experiments including retroviral injections. Additionally, I will explore what effects Nrxn1 cKO has on the niche astrocytes themselves through 3D reconstructions and detailed morphological analysis. Lastly, I will investigate, through electrophysiological recordings, how the removal of Nrxn1 in niche astrocytes affects the integration and function of newly generated neurons in the DG, and how this affects overall circuit function/plasticity.

Characterizing the effect of synuclein-mediated microglia stimulation on human neuronal cells

Leonore Düfel[1], Jonas Lanfer[1], Laura Krumm[1], Tom Börstler[1], Alice Drobny[2], Fanny Boros[2], Martin Regensburger[1,2], Friederike Zunke[2] and Beate Winner[1]

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Parkinson´s disease (PD) with its leading symptoms of tremor, rigor and akinesia is a common neurodegenerative disease whose pathogenesis still leaves many questions unanswered. A pathological hallmark of PD is the accumulation of high molecular weight alpha-synuclein-fibrils and the degeneration of dopaminergic neurons. Several studies suggest that microglia as immune cells of the CNS may play an important role in the pathogenesis of PD. Preliminary results from our lab demonstrated that alpha-synuclein-fibrils are able to induce a robust inflammatory response in human Microglia-like-cells
(iMGL). However, the specific influence of alpha-synuclein-mediated stimulation of microglia on human neuronal cells still poses an unanswered question.
In this project, we aim to address this question using human induced pluripotent stem cell (iPSC) based models.

Absence of the RING domain in MID1 results in severe patterning defects in the developing human brain

Sarah Frank

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The X-linked form of Opitz BBB/G syndrome (OS) is a monogenic disorder in which symptoms are established early during embryonic development. OS is caused by mutations in MID1 scattered throughout the whole gene locus except in the N-terminal RING domain harboring the ubiquitin ligase activity. By employing genome-edited human induced pluripotent stem cell (hiPSC) lines, we here show that mutations in this N-terminal RING domain of MID1 causes severe patterning defects in human brain organoids. We observed a prominent neurogenic deficit with a reduction of neural tissue and a concomitant increase in choroid plexus structures. Expression analyses revealed a deregulation of the patterning pathways SHH, WNT and BMP very early on, even prior to neural induction. Strikingly, these phenotypes are in contrast to MID1 full-knockout organoids suggesting gain-of-function mechanisms underlying the patterning defects. The severity and early establishment of the phenotype could serve as a likely explanation for the absence of patients with mutations the N-terminal RING domain of MID1.

The role of the Ep400/Tip60 chromatin remodeling complex in the cranial neural crest and orofacial clefting

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The cranial neural crest plays a fundamental role in orofacial development and morphogenesis. As a pluripotent and dynamic cell population, the cranial neural crest is undergoing vast transcriptional alterations throughout embryogenesis and the formation of facial structures. These transcriptional changes are regulated by several transcription factors and remodeling complexes. Previously, we revealed the relevance of the Ep400/Tip60 histone acetyltransferase complex in the cranial neural crest and that a knockout of Ep400 causes neural crest-related malformations such as orofacial clefting (Fröb et al. 2019). Furthermore, a case study identified three patients carrying missense mutations in Tip60 who showed severe mental impairments as well as orofacial clefts (Humbert et al. 2020). The exact molecular causes and mechanisms, however, are still unknown.
In this study we selectively knocked out Ep400 or Tip60 in the murine cranial neural crest cell line O9-1 to examine its roles in neural crest biology. To understand the regulatory effects of Ep400 and Tip60, knockout neural crest cells were investigated by bulk RNA sequencing to unravel transcriptomic changes in the affected cells. Bioinformatic analyses hinted at the regulation of major cellular functions such as proliferation, ATP generation and protein synthesis by the Ep400/Tip60 complex.
Reduced proliferation was confirmed by crystal violet staining, phospho-histone H3 staining and the determination of mitotic cells with condensed chromatin in vitro. We did not detect increased apoptosis in the knockout cell lines. The energetic profile of the cells was investigated by Seahorse technology. The ATP-rate assay showed a decreased glycolytic activity in Ep400- or Tip60-deficient cells. An O-propargyl-puromycin (OPP) Click-iT assay revealed a significant reduction in protein synthesis. To verify in vivo the discovered in vitro effects, Ep400 and Tip60 were selectively ablated in cranial neural crest using Wnt1-Cre in transgenic mice. The knockout of each of the subunits resulted in severe craniofacial malformations from E12.5 onwards. At E10.5 a significant reduction in neural crest-derived tissue and proliferation rate was evident.
The strong defect in orofacial structures of mice lacking Tip60 or Ep400 completely correspond to the milder orofacial malformations in patients carrying heterozygous missense mutations. Our results furthermore argue that changes of metabolism, protein synthesis and proliferation in cranial neural crest cells are responsible for the orofacial defects observed in patients.

Analysis of ectopic Sox9 expression during different timepoints of oligodendrocyte development in the murine CNS

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Repair processes in the diseased or injured adult brain are often dependent on the reactivation of processes that are usually active during ontogenetic development. In the case of demyelinating diseases such as Multiple Sclerosis, repair requires the generation of new oligodendrocytes and myelin by NG2 glia. Characteristically, early postnatal NG2 glia proliferate and differentiate quite efficiently as well as express Sox9 endogenously. On the other hand, adult NG2 glia do not maintain this efficient differentiation ability and, interestingly, do not express Sox9. Therefore, we investigate whether this difference in Sox9 expression could be the key to more easily differentiating adult NG2 glia. We show that the overexpression of Sox9 in developing and early postnatal NG2 glia leads to an increase in their ability to proliferate and differentiate. We also show that ectopic Sox9 expression in adult NG2 glia causes these cells to differentiate more efficiently and effectively. Sox9 could be one of the key regulators in NG2 glia differentiation, opening the door to future therapies in demyelinating diseases.

The role of Sox8 and Sox10 for myelin maintenance

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Multiple sclerosis (MS) is one of the most common neurodegenerative diseases affecting approximately 250.000 adults in Germany. While the disease is well-understood in terms of progression and pathology, little is known about its development. Although many risk factors have been identified, for some the exact connection to disease onset remains unclear. One of these factors are SNPs in close proximity to Sox8 found in a wide screening of MS patients. The protein function of Sox8, however, has not yet been connected to the pathogenesis of MS.
Previous work has shown that the transcription factor Sox8 shares its role in developmental myelination with the closely related transcription factor Sox10. Since Sox10 plays the major role, knockout of Sox8 does not lead to a strong myelination defect in mice. In cell culture, Sox8 seems to regulate many inflammatory genes as revealed by RNA sequencing. This leads to the hypothesis that Sox8 is relevant for maintenance and protection of myelin sheaths by inhibiting inflammation.
In this study we selectively knocked out Sox8 and Sox10 either simultaneously or independently in the adult murine central nervous system (CNS) to examine its role in myelin maintenance in the mouse brain. Total oligodendroglial numbers were not decreased in any mouse mutant. The population of oligodendrocyte precursor cells also did not change after deletion of the respective Sox genes. Furthermore, proliferation and cell death markers showed no phenotype in single- or double knockouts. Numbers of the Myrf-expressing cells were reduced in Sox10-deficient animals as well as in the double-knockouts, likely due to the direct regulation of Myrf by Sox10. On RNA level a reduction of myelin gene expression could be seen in the Sox10 knockout and in double knockout animals.
So far, no clear phenotype linked to loss of Sox8 in the adult CNS could be seen. To obtain a better understanding of Sox8 function, ultrastructural analysis of brain samples via electron microscopy are ongoing. Additionally, RNA sequencing of Sox8-deficient oligodendrocytes will be performed to identify Sox8 target genes and its regulatory network.

Involvement of microglia in Hereditary Spastic Paraplegia type 11

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Neuroinflammation, and microglia, in particular, have emerged as key players in the pathogenesis of neurodegenerative and motor neuron diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). In a recently published mouse model of another rare motor neuron disorder, Hereditary Spastic Paraplegia type 11 (SPG11-HSP), pathogenic neuroinflammation including microgliosis was also found in distinct compartments of the diseased CNS. SPG11-HSP is a complex form of HSP that is caused by pathogenic variants in the SPG11 gene. The disease is characterized by progressive weakness and spasticity of the lower limbs besides additional neurological symptoms such as thinning of the corpus callosum and cortical atrophy. Here, we demonstrate for the first time an inflammatory pathology in a human model of SPG11-HSP. Analyzing the serum of SPG11-HSP patients, elevated levels of proinflammatory resistin and reduced levels of the important immunomodulator TGFβ1 were revealed in comparison to healthy controls. To investigate if microglia that lack SPG11 encoded spatacsin are affected in a cell-autonomous way, we aimed to functionally characterize iPSC-derived microglia-like cells (iMGLs) from three SPG11-HSP patients (SPG11-iMGL). SPG11-iPSC differentiated equally into iMGL in comparison to control iMGL and did not feature any lipid or lysosomal accumulations. However, all SPG11-iMGLs did produce and secrete more proinflammatory cytokines like IL-1β and TNFα specifically upon IFNγ treatment, indicating an over-active microglial stage. Although the exact correlations still need to be determined, we propose that neuroinflammation is contributing to SPG11-HSP pathology and that microglia are affected in a cell-autonomous way. To further validate our hypothesis, postmortem brain tissue from SPG11-HSP patients will additionally be examined for microglia-associated pathology. Clarifying the precise role of the immune system in SPG11-HSP brings us closer to uncovering the pathological mechanism and finding therapeutic approaches.

Investigating BACE1's role in peripheral sensory processing

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The β-site APP cleaving enzyme1 (BACE1) accounts for the rate limiting step in the production of toxic β-amyloid in Alzheimer's disease (AD). Besides intense research about BACE1's role in the pathogenesis of AD, many efforts have been put into unraveling physiological functions of the β-secretase over the last years. Increasing evidence suggests an involvement of BACE1 in peripheral sensory processing, including proprioception, pain and thermosensation. BACE1-/- mice have been reported to exhibit altered thermal thresholds (hot), a decreased number and impaired formation of muscle spindles, as well as deficits in myelination in peripheral nerves. To further investigate sensory afferents in BACE1 -/- mice, we recorded action potentials of isolated dorsal root ganglia (DRG) neurons, which showed higher frequency of action potential firing, increased current thresholds and reduced amplitudes. We then mechanically stimulated DRG neurons to evoke mechanosensitive currents. Our data demonstrated that BACE-/-DRG neurons with high currents exhibited reduced latencies in response to mechanical stimulation. Meanwhile, Piezo1 and 2 currents were not significantly altered in the presence of co-expressed BACE1 in transfected HEK cells, although minor reduction of current amplitude and increase of decay time were observed. Finally, preliminary behavioral experiments showed that BACE-/- mice have reduced mechanical withdrawal thresholds (v. Frey test) indicating an increased sensitivity to mechanical stimulation compared to wild type littermates. Our data reinforces the secretase's contribution to peripheral sensory processing and suggests an involvement of BACE1 in mechanosensation. To provide further insights into BACE1's role in peripheral somatosensation, we will perform comprehensive sensory testing of BACE-/- and wild type mice and investigate nerve morphology, mechanosensor composition and distribution in the skin.

Modeling Sox11-related Coffin-Siris Syndrome in human embryonic stem cells Institüt fur Biochemie

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SOX11 is a transcription factor that has been described as an important regulator of stem cell behavior and neurogenesis. In mice, SOX11 acts redundantly with members of the SOXC family, SOX4 and SOX12. Surprisingly, in humans, missense mutations or deletions in the SOX11 gene have been causally linked to “Coffin-Siris Syndrome like Syndrome (CSS9)” a congenital neurodevelopmental disorder with symptoms including intellectual disability, microcephaly, and autism spectrum disorder like pathology. The fact that the other members of the SOXC family cannot compensate for these mutations suggests that SOX11 has evolved differently in humans, acquiring an important role in human neural development.

Our group was the first to generate an in vitro model to study the role of SOX11 in neural development, using hESCs with a missense mutation in a SOX11 allele. Preliminary data from differentiation protocols showed an imbalance in the generation of GABAergic interneurons and glutamatergic excitatory neurons as well as a pathological electrophysiological development of neural networks. In addition, bulkRNA sequencing indicated disturbances in pathways related to dorsal/ventral patterning formation, neurogenesis, glutamatergic and GABAergic synapse formations, and neurogenesis.

In order to understand the developmental role of SOX11 in interneuron formation, I will employ SOX11 haploinsufficient hESCs to generate organoids, the current state of the art in recapitulating development, to investigate if SOX11 is interfering within the different developmental stages of neuron/interneuron formation and dorsal/ventral patterning. Furthermore, I would like to perform ChIP-seq to evaluate the possible molecular targets of SOX11. Together this finding would help better understand the disease mechanisms of CSS9 as well as elucidate the role of SOX11 in human development.

The interplay of pro-inflammatory cytokines and alpha-synuclein in neuronal cytoskeleton pathology

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Neurons express a range of cytokine receptors, rendering them vulnerable to neuroinflammation and neurodegeneration. It is generally accepted that inflammation impairs neuronal structure and axonal transport, and evidence is emerging that pro-inflammatory cytokines such as TNF-α can disrupt microtubules and detach molecular motors from the cytoskeleton in a variety of cells. However, it remains unknown how specific cytokines or their combinations compromise neuronal physiology through their effects on neuronal cytoarchitecture, particularly in the context of neurodegeneration.
Here we report that T cell-derived pro-inflammatory cytokines IL-17A, TNF-α and IFN-γ, or a combination thereof, impair axonal transport and destabilise the cytoskeleton in human neurons. The induced pluripotent stem cell (iPSC)-derived cortical neurons expressed a range of cytokine receptors whose levels were dysregulated in neurons harbouring a duplication of the α-synuclein gene (SNCA dupl), which is implicated in Parkinson's disease. These cells also differentially regulated receptor expression upon cytokine application. Using microfluidics, we observed that cytokine treatment reduced mitochondrial speed and shifted the directionality of mitochondrial movement towards retrograde transport, and that this pathology was enhanced in SNCA dupl neurons. Further work will determine the impact of the cytokine–α-synuclein interplay on specific cytoskeletal components and signalling pathways.
Our work delineates the detrimental effects of pro-inflammatory cytokines on human neurons and their possible interaction with alpha-synuclein, highlighting a potential contribution of inflammation-mediated cytoskeleton disruption to neurodegenerative disorders.

Direct lineage reprogramming as a heuristic approach to identify key players in human neurogenesis

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Adult human brain derived pericytes are amenable to direct lineage reprogramming via overexpression of Ascl1 and Sox2 thereby converting into electrophysiologically active neurons. Molecular dissection of the reprogramming trajectory by scRNAseq revealed that cells changing their identity pass through an intermediate state transcriptionally reminiscent to neural stem cell (NSC) in the developing brain. We hypothesize that genes induced along the process of cellular identify change towards induced neurons constitute a distillate of molecular key-players crucially involved also in neuron formation during human brain development. The aim of our project is to assess the role of candidate genes predicted to drive the transition of pericytes undergoing reprogramming through the NSC-like state in human developmental neurogenesis. We will employ a system allowing inducible genetic modifications in human brain organoids to characterize the role of these candidates during neural development, potentially giving new insights in human-specific aspects of neurogenesis. Dissecting common molecular mechanisms between natural and forced neurogenesis represent a novel approach to understand how human neurons are generated.

Sphingomyelin synthases in depression and antidepressant treatment

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Major depressive disorder is a severe and common psychiatric disease. Recent studies in mice have revealed that pharmacological inhibition of sphingomyelin synthases (SMS) induces neurogenesis and rapidly improves depression-like behaviour by activating autophagy. However, whether these effects are due to the inhibition of one or both SMS isoforms (SMS1 and SMS2) remains unknown. We aim to elucidate the involvement of SMS1 and SMS2 in depression and to identify their role in the autophagy-dependent antidepressant effect. Therefore, we have compared the activity and expression of SMS under human healthy and pathological conditions. Our first data indicate a sex-independent higher expression of SMS genes in peripheral blood cells from patients with a current major depressive episode (n=129) compared to healthy controls (n=60). In addition, an increase of SMS enzyme activity was observed in female but not male depressed patients compared to controls. To study the specific roles of SMS isoforms on autophagy, we downregulated SMS1 or SMS2 with siRNAs in H4 cells. The *in vitro* studies showed that downregulation of SMS1 (but not SMS2) leads to an accumulation of the autophagic marker LC3B-II in the presence of bafilomycin A1 compared to control cells, indicating an increased autophagic flux. Moreover, the expression of several autophagy related genes (*SQSTM1*/p62, *ULK1*, *PIK3C3*) is increased specifically after SMS1 downregulation. Overall, our results fit with the hypothesis of increased SMS activity in depression and with a specific involvement of SMS1 in autophagy induction, thereby suggesting a potential pharmacological target for the design of fast-acting antidepressants.

Effect of methanol fixation on single cell RNA sequencing of the murine dentate gyrus

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Droplet-based single-cell RNA sequencing provides a powerful tool to evaluate the transcriptomic landscape and heterogeneity of thousands of cells in parallel. However, complex study designs or the unavailability of in-house instruments require the temporal disconnection between sample preparation and library construction, raising the need for efficient sample preservation methods which are compatible with downstream applications. Here, we evaluated the suitability of cryopreservation and methanol fixation for preserving single cell suspensions of the murine dentate gyrus.
To evaluate the performance of both methods, we first evaluated the recovery after thawing and rehydration of the papain-dissociated cell suspensions. Due to the low recovery rates after cryopreservation, further evaluation steps were focused on methanol fixation. Its efficacy was determined via flow cytometry of Sytox-stained samples. RNA quality was assessed on an Agilent bioanalyzer. To test whether and how methanol fixation affects transcriptome profiling, we performed a SORTseq experiment on fresh and methanol-fixed cell suspensions.
Methanol fixation resulted in higher recovery rates than cryopreservation. It had an efficacy of 100% and no effects on RNA integrity. Transcriptome analysis revealed that methanol fixation results in a slight drop in read and gene counts, suggesting RNA leakage. However, it did not interfere with clustering and cell-type composition. Moreover, it resulted in more high-quality cells and reduced signs of mitochondrial stress compared to fresh samples. Together, these data suggest that methanol fixation is suitable for storing neural cells for subsequent single-cell RNA profiling, helping to overcome challenges arising with complex workflows and to improve the experimental flexibility.

Lysosomal acidification and function is modulated by FoxO-signaling in adult hippocampal neural/stem progenitor cells

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Ensuring adult neural stem cell function in the mammalian brain over time is controlled by niche derived factors and stem cell's endogenous transcriptional programs. Furthermore, studies from the last decade have shown that the metabolic system and the proteasome and autophagy-lysosome pathway (ALP) system play a crucial role in adult neural stem cell homeostasis and maintenance of adult neurogenesis. The mechanisms controlling ALP activity in adult neural stem cells and subsequently generated neurons are largely unknown. We recently identified transcription factors of the FoxO family as central regulators of ALP activity in adult hippocampal neurogenesis with ramifications for neural stem cell homeostasis and for morphology of newborn neurons. Our new findings in FoxO-deficient adult hippocampal neural stem/progenitor cells indicate insufficient acidification of lysosomes, which in turn leads to the accumulation of fused autolysosomes and the blockage of the following degradation process. The lysosomal pH is regulated by the vacuolar ATPase (v-ATPase), a multi-subunit proton pump located in the lysosomal membrane, which pumps protons into the lysosomal lumen under ATP hydrolysis upon its reversable assembly. We identified subunits of the v-ATPase as well as modulators of both v-ATPase function and lysosomal enzymes as potential targets of FoxO signaling.
Our recent findings suggest a new link between FoxO transcription factors and v-ATPase-dependent lysosomal acidification and function in adult neural stem/progenitor cells.

Dysregulated homeostasis of astrocytes contributes to the pathogenesis in a MSA mouse model

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Astrocytes are macroglial cells populating the whole central nervous system (CNS) and fulfill a plethora of complex functions. The capability of astrocytes to adopt to exogenous conditions is referred to as 'reactive astrogliosis'. The involvement of astrocytes in neurodegenerative processes heightened the need for identifying distinct molecular markers for defining their contribution to disease progression. A rare atypical parkinsonian disorder is multiple system atrophy hallmarked by glial cytoplasmic inclusions of α-synuclein, neuroinflammation, neuronal loss as well as myelin loss. Here, we take advantage of a mouse model of multiple system atrophy (MSA) featuring an oligodendrocyte-specific (MBP29) overexpression of human α-synuclein. In addition, we optimized isolation of astrocytes expressing ATPase Na+/K+ Transporting Subunit Beta 2 using magnetic activated cell sorting for bulk mRNA sequencing. Our findings provide evidence on protein and transcriptional level for a region-specific astrocytic response in the present MBP29 model. More than 1000 genes are differentially regulated in the MSA mice, among others a strong upregulation of a variety of reactivity markers such GFAP, Vimentin, CD44, Osmr, and Serpina3n. Based on an observed decrease of glutamate reuptake transporter expression, we propose an impaired glutamate clearance in the striatum, the most important relay for motoric signals in the CNS. Using gene set enrichment analysis, we also provide evidence of inflammatory processes and impaired lipid metabolism, as well as dysfunctional cholesterol homeostasis in the brain of MSA mice. Together, these findings imply a significant involvement of astrocytes in MSA pathogenesis by profound change of homeostatic functions of astrocytes.

Nup153 regulates activity dependent-gene expression in neurons

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Neurons respond to environmental stimuli and exhibit functional and structural plasticity. In response to synaptic inputs, neurons induce activity-dependent gene expression (ADE) programs, which underlie neural plasticity and memory. Importantly, to maintain spatiotemporal accuracy of neuronal plasticity, the balance between induction and repression of ADE is crucial. ADE should be tightly repressed before neuronal activation, but have to be induced immediately when neurons are stimulated. Mysregulation of ADE could lead to several neurodevelopmental and neurological disorders. Although molecular mechanisms inducing ADE upon neuronal activation have been intensively investigated, on the other hand, mechanisms underlying the repression of ADE in neurons have remained largely elusive.

In the past decade, nuclear pore proteins (nucleoporins, Nups) have been linked with cell type-specific gene regulation, in addition to their classical roles in nucleocytoplasmic transport. Especially, recent studies found that Nup153, one of nuclear basket protein, regulates epigenetic programs to maintain neural progenitor cells. Interestingly, we found that the levels of Nup153 is dynamically regulated among difference neural cell types, and it is highly expressed in mature neurons. We thus hypothesized that Nup153 has specific roles in neuron-specific genetic programs such as ADE. To address roles of Nup153 in mature neurons, we performed loss- and gain-of-function of Nup153 and found that Nup153 is necessary and sufficient for repressing ADE. Our epigenetic profiling further indicated that Nup153 could directly repress ADE. Our data suggests Nup153 acts as a repressor of ADE, thus Nups may work as structural platform to tightly regulate ADE.

Proliferation vs. differentiation – a function for the C-terminal binding protein 1 in mouse retina development?

Julia von Wittgenstein

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The retina is a particularly heterogeneous neuronal network, which comprises at least 130 different types of neurons in mice. This is a prerequisite for efficient processing of the complex visual information. During development, these neurons derive from a single type of neuroepithelial cells in a complicated lineage with multiple neuronal types emerging at the same time. Until now, knowledge is sparse on how the temporal sequence of neuron births and progressive lineage restriction of the retinal progenitors are achieved.

Here, we investigated whether the transcriptional co-repressor and synaptic protein CtBP1 is one of the transcriptional regulators for neuronal type specification in the retina. In mouse embryonic and postnatal retina, we found high expression levels of CtBP1 in postmitotic, fate-determined immature neurons. In contrast, proliferating neuroblasts displayed only low levels of CtBP1. Deletion of CtBP1 in CtBP1 knockout mice was compatible with an overall intact formation of the retinal cell layers in most KO animals. However, the early postnatal retina of some KO mice harbored an accumulation of proliferating, undifferentiated cells. These findings pointed towards a function of CtBP1 in cell cycle exit and fate-determination of retinal neurons. Accordingly, we observed a delayed functional maturation of the retinal network in electroretinogram recordings of CtBP1 KO mice.

Activating ß-glucocerebrosidase by exploiting its transporter LIMP-2

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Mutations in the GBA gene cause of the lysosomal storage disorder Gaucher disease and are among the highest genetic risk factors for the development of the neurodegenerative disorder, Parkinson disease (PD). GBA encodes the lysosomal enzyme beta-glucocerebrosidase (GCase), which orchestrates degradation of glucosylceramide (GluCer) in the lysosome. Impaired GluCer metabolism is associated with neuronal damage and neurodevelopmental defects. Recent studies have shown that GluCer accelerates alpha-synuclein aggregation, exposing GCase as a promising target in the treatment of PD. Ongoing research and clinical trials are aiming to identify GCase-activating compounds for clinical application.

In this study, we investigated the interaction of the lysosomal membrane protein and GCase transporter LIMP-2 with wild type (wt) GCase and the most prominent disease-associated GCase variants: E326K, N370S and L444P. Our data reveal that LIMP-2 not only mediates transport of GCase to the lysosome, but functions as a potent allosteric activator of the enzyme. Overexpression of LIMP-2 boosted lysosomal transport of GCase and rescued activity of the E326K variant in HEK293T cells. We found that a single helix in the LIMP-2 ectodomain was sufficient for activation of GCase in vitro. Based on this helix, we designed a custom lysosome-targeted peptide to restore lysosomal GCase activity in fibroblasts of PD patients harboring GBA E326K mutations. These findings expose the LIMP-2 interaction site on GCase as a potential therapeutic target to rescue lysosomal GCase activity, thereby revealing a novel approach for the design of GCase-activating compounds.

Poster Session 2 – Thursday Oct. 6th , 10:00 – 12:00 am

Two-faced effect of CSF1R-mediated myeloid cell depletion in a model of multiple system atrophy

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As CNS-resident macrophages, microglia fulfill numerous functions important for brain development and homeostasis. In the context of neurodegeneration, they have been implicated in de- and regenerative processes. However, distinct activation patterns including increased phagocytosis indicated a damaging role of microglia in multiple system atrophy (MSA), a devastating, rapidly progressing atypical parkinsonian disorder which still lacks causal therapy. Here, we analyzed the gene expression profile of microglia in a MSA mouse model and identified a disease-associated expression profile with an upregulation of the colony-stimulating factor 1 (Csf1). Hence, we hypothesized that the disease progression and neuropathological phenotype can be modified by CSF1 receptor-mediated depletion of myeloid cells, predominantly microglia. Interestingly, myeloid cell depletion revealed a two-faced effect in MSA mice comprising an increased lifespan accompanied by a delayed onset of neurological symptoms in contrast to severely impaired motor functions. Moreover, PLX5622 reversed gene expression profiles related to disease-associated microglia, however, reduced gene expression associated with synaptic function. While these transcriptional changes were accompanied by a reduction of dopaminergic neurons in the substantia nigra pars compacta, an increase of the striatal neuritic density was observed upon myeloid cell depletion in MSA mice. Overall, our findings provide insight into the complex, two-faced role of myeloid cells in the context of MSA comprising not only an inflammatory role, but also a supporting role for neuronal function. Thus, this study emphasizes the importance to carefully balance the beneficial and adverse effects of CSF1R inhibition in different models of neurodegenerative diseases prior to its clinical translation.

To die or not to die - does loss of bassoon play a role in cone photoreceptor survival?

Miriam Ryl

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The presynaptic protein Bassoon (BSN) is an important component of the active zone of chemical synapses, where it contributes to synapse assembly and function. Recent studies show a novel function of BSN as an inhibitor of presynaptic autophagy and proteasomal degradation in brain neurons.
In the retina of two BSN-deficient mouse lines, BsnΔEx4/5 and Bsngt, we found degeneration of cone photoreceptors and retinal remodeling. This raises the question of whether BSN is important for the survival of these highly active sensory neurons by controlling homeostasis pathways. Interestingly, we did not find such a retinal phenotype in a third BSN-deficient mouse line (Bsnko). Our recent results show that proteasomal degradation is disrupted in the retina of both Bsngt and Bsnko mice, thus changes in protein degradation alone is not sufficient for cone photoreceptor degeneration. We hypothesize that an additional trigger is required.
The trigger for the degeneration of the cone photoreceptors could be the presence or absence of certain BSN regions in the form of a residual BSN fragment. We will examine these possibilities by crossing the different BSN-deficient mouse lines. This may help to determine which regions of BSN are critical for cone photoreceptor survival and which can be compensated by other proteins.
In summary, our data suggest that BSN plays an important role in processes of cellular homeostasis and cone photoreceptor survival in the retina.

Temporal patterning by POU transcription factors defines neuronal subtypes of the Anterolateral System

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Neuronal diversity emerges from the superposition of spatial and temporal patterning programs in large regions of the developing nervous system. This is exemplified in the spinal cord, where distinct classes of neurons, generated from spatially segregated progenitor domains, are partitioned into molecularly and functionally distinct subtypes by a shared temporal patterning program. While the gene regulatory network (GRN) orchestrating spatial patterning is well-understood, the GRN architecture controlling temporal patterning is just emerging. Here, we show evidence of a temporal program driving neuronal subtype specification of anterolateral system (AS) neurons that relay somatosensory information from the spinal cord to the brain. We describe an early emergence of transcriptomic differences between AS neuron subtypes expressing the POU transcription factors Pou2f2 and Pou3f1, and show that these subtypes occupy distinct positions in the developing spinal cord. Using a birth-dating approach, we subsequently demonstrate that Pou2f2 and Pou3f1- positive AS neurons are generated at distinct stages during development and that Pou3f1 acts as a temporal transcription factor in other classes of spinal cord excitatory neurons. Together, our results demonstrate that the molecular and functional identity of AS neurons is specified early by a global temporal program.

Leveraging direct neuronal reprogramming to identify novel key players in human neurogenesis

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For many years advances in the study of human CNS development and function have been hindered by the inaccessibility of human tissue and hence the molecular basis of neuronal subtype specification and formation in mammals largely stems from studies in rodent models. Albeit similar, the human brain differs in many aspects from that of rodents. Direct lineage reprogramming of terminally differentiated somatic cells into induced neuronal cells (iNs) opened up the possibility to shed light on the molecular mechanisms driving human neurogenesis. Among the somatic cells amenable for iN reprogramming, pericytes, naturally residing within the human brain, represent an ideal candidate. Indeed, it has been demonstrated that, via retroviral overexpression of Ascl1 and Sox2 (AS) in pericytes derived from the adult human cerebral cortex, it is possible to obtain actively firing neurons. However, the key molecular nodes that orchestrate this reprogramming trajectory have not been explored yet. Here, we use pericyte-to-iN conversion as a tool to identify genes potentially involved in human neuron formation by performing high resolution molecular analysis of the conversion process on a single cell level. Deconstructing the reprogramming process by simultaneous profiling of the transcriptional and the chromatin accessibility changes in the very same cells, allows both to formulate hypotheses on the molecular nature of reprogramming barriers and target cell subtype-specification. Thus, we speculate that molecular key nodes responsible for acquiring neuronal identity during direct lineage reprogramming are also key in developmental neurogenesis. Ultimately our aim is to investigate whether and to what extent such forced neurogenesis recapitulate the naturally occurring one and extract the key essence of becoming a human neuron.

Possible role of NKCC1 in the fate of hippocampal neural stem cells during Alzheimer's disease

Anna-Lena Fleischer

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Alzheimer´s disease (AD) is one of the main neurodegenerative disorders of the 21st century, characterized by a progressive decline in cognitive function as manifested by a difficulty in learning new information and memory loss. Currently, AD is considered incurable and barely treatable, leading to an increasing need for patient care, posing enormous social and economic challenges to society and healthcare systems worldwide.

One important mechanism in memory formation and learning is adult neurogenesis, the process by which new neurons arise from neural stem cells in the dentate gyrus of the adult hippocampus. Current research of our group suggests that neural stem cells are affected in their fate decisions by AD. We observed an increased proliferation of neural stem- and progenitor cells in the pre-plaque phase which is followed by a strong decline in the number of stem cells and adult neurogenesis, and an increased astrogenesis. Factors modulating neural stem cell proliferation during adulthood and ageing are not well understood, however, our own preliminary data suggest that the loss of the co-transporter NKCC1 in stem cells alters proliferation behavior. The cation-chloride co-transporters NKCC1 and KCC2 are important modulators of intracellular chloride homeostasis and thus of the GABAergic system. The aim of the present study was to investigate the underlying mechanisms of early-onset of stem cell proliferation in AD. To this end, we quantified the expression levels of NKCC1 and KCC2 in hippocampal neural stem cells in young and middle-aged Nestin-GFP mice and APPxPS1/Nestin-GFP triple-transgenic mice, using confocal microscopy and 3D reconstruction with Imaris software. During ageing, levels of the NKCC1 co-transporter significantly decreased in the Nestin-positive stem cells. Importantly, this age-related decrease was already seen in the Alzheimer's animals in the pre-plaque phase. On the contrary, in the aged stem cells the KCC2 co-transporter was increased. Our results suggest that alteration of NKCC1 and KCC2 play an important role in the early overactivation of adult neural stem cells during Alzheimer's disease.

Uncover common and diverging mechanisms underlying aberrant formation of long-range neuronal connections in neurodevelopmental diseases

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The establishment of long-range connections in the human brain is a complex process involving distinct differentiation cues along neurodevelopment. The lack or misconstruction of long-range connections can be attributed to various factors and can lead to a multitude of neurodevelopmental and neuropsychiatric disorders. In this project, we focus on three neurodevelopmental disorders, Optiz BBB/G syndrome, caused by mutations in the gene encoding for the microtubule binding protein MID1, Coffin-Siris syndrome, which can be caused by mutations in the SOX11 gene encoding for a transcription factor, and Pitt-Hopkins syndrome, caused by mutations in the gene encoding the transcription factor TCF4. In all three disorders the formation of long-range neuronal connections over the corpus callosum (CC), a structure critical for the communication between the two cortical hemispheres, is impaired, but the cellular and molecular mechanisms responsible for the malformation of long-range connections are yet to be defined. Therefore, the aim of this project is to dissect underlying mechanisms and reveal common and diverging key-nodes driving the aberrant development of cortical projection neurons in these phenotypically related but genetically distinct syndromes.

Unraveling the gut-brain axis in Parkinson´s disease using iPSC-derived models of enteric and central nervous system

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Parkinson's Disease (PD) is a common neurodegenerative disorder characterized by symptoms such as tremors and movement impairments along with non-motor manifestations including psychiatric, cognitive, and autonomic dysfunctions [1, 2]. Genetic cases usually account for 10% of PD manifestations and among them are those related to triplications in the SNCA gene, which encodes for -synuclein, one of the key proteins related to PD pathology [2]. It has recently been proposed that PD may start in the gut, in a way that ingestion of environmental pathogens induces -synuclein accumulation in the bowel and then the α-synucleinopathy reaches the Substantia Nigra via the nigro–vagal pathway, inducing the degeneration of dopaminergic neurons [3]. Accordingly, it was already shown that PD patients show accumulation of -synuclein in the bowel during the pre-clinical phase of the disease [4]. In this sense, we believe that characterizing this gut-brain axis may open new therapeutic avenues for PD patients in the near future.
Hypothesis: We hypothesize that the SNCA triplication mutation in PD will lead to accumulation of -synuclein in the iPSC-derived enteric nervous system (ENS) cells. Consequently, this should affect the transcriptional signature at a single cell level, along with the neuronal activity, biomechanics, cellular metabolic profile and immunogenic potential. Moreover, we believe that the -synucleinopathy can propagate from the ENS to the central nervous system (CNS), leading to dopaminergic neuron death.
Objective: To investigate if the SNCA mutation leads to accumulation of -synuclein in the ENS; if accumulated -synuclein can propagate from the ENS to the CNS and subsequently the transcriptional, mechanical, metabolic and inflammatory alterations that could be related to this phenotype.
Preliminary Results: Our group has obtained successfully iPSC-derived ENS lines and our preliminary results demonstrate that these cells are capable of responding to proinflammatory stimuli.

Effect of NDR2 on circadian regulated Autophagy in the dorsal Hippocampus

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The serine-threonine-kinase NDR2 is part of the hippo-pathway and it regulates neuronal growth, spine formation, arborization, and recently it has been implicated in the positive regulation of neuronal autophagy. Given that autophagy is under circadian control through the mTOR pathway, we explored the possible regulation of hippocampal autophagy by NDR2 in a circadian fashion. Therefore, hippocampal brain samples from NDR2 overexpressing mice were taken at 2 different time points; one in the inactive phase (ZT 7) and another in the active phase (Zeitgeber time 15) which correspond to the peak and the lowest level of basal neuronal autophagy, respectively. Then, the status of mTOR-signaling and autophagic flux was evaluated by immunoblot analysis. We showed that during the active phase in the overexpressing mice, there is an increase of the p-T202/204-ERK (T185/187 of Erk2)/ERK ratio that correlates with an increased Intensity of p-ERK in the granular cells, and a disruption in the circadian phosphorylation of p-S2448-mTOR. Furthermore, in the inactive phase, we observed an increase in LC3 and p62 levels in the dorsal hippocampus as well as increased p62 puncta in the CA3 and CA2 regions. Overall, NDR2 overexpressing mice show disruption in the circadian-regulated mTOR signaling in the active phase with increased levels of autophagic proteins in the inactive phase, which suggests a diminish of the autophagic flux. These results indicate that NDR2 might lie in between the circadian regulation of autophagy through mTOR signaling.

Tissue mechanics-modulating small leucine-rich proteoglycans contribute to the differential regenerative capacity of mammalian and zebrafish central nervous system axons

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Spinal cord injury results in the formation of fibrous scar tissue in mammals. The scar forms through deposition of extracellular matrix (ECM) and constitutes a hostile environment to axon regeneration, thereby preventing recovery of function. In contrast, the ECM in the spinal lesion site of zebrafish is permissive and required for regeneration. Whether interspecies differences exist in the composition of the injury ECM that contributes to regeneration failure and success has, however, not been systematically addressed.

We performed cross-species comparative proteomics to identify differences in the ECM composition of spinal cord-lesioned zebrafish and rat. Differentially enriched candidate proteins were analyzed for axon growth-modulating properties, using a range of in vivo and in vitro assays.

We identified members of the small leucine-rich proteoglycan (SLRP) family to exhibit high abundance in rat and human central nervous system (CNS) lesions but not in regenerative-competent zebrafish. Experimentally increasing the protein levels of individual SLRPs in the zebrafish lesion environment inhibits axon regeneration and functional recovery. Mechanistically, we obtained evidence that SLRPs modulate the mechanical properties of the lesion environment toward a non-permissive signature.

Our data identified SLRPs as previously unrecognized axon growth-limiting ECM components that contribute to the differential regenerative capacity of mammalian and zebrafish CNS axons, and indicate an impact of tissue mechanics on the regeneration outcome.

Human dorsal forebrain organoids help to elucidate cell type-specific effects of maternal immune activation on neocortical development

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Prenatal exposure to maternal immune activation (MIA) during the first trimester of gestation is correlated with long-term deficits in brain development in the offspring, including increased risks for autism spectrum disorder (ASD) in humans. Rodent models show causal links between prenatal exposure to MIA and ASD-like behavioral deficits in pups that are mediated by deficits in protein translation in neurons. While the majority of studies on the effects of MIA focus on neuronal abnormalities, a growing body of evidence suggests that defects in proliferative and neurogenic capacity of neural stem cells may be important contributors to ASD. In order to determine changes in neurogenesis following MIA in human neural stem cells, we treat human iPSC-derived dorsal forebrain organoids with molecular mediators of MIA. We have validated our model by showing that both dorsal forebrain organoids and midgestational human neocortex express the molecular machinery necessary for the response to the mediators of MIA. We have investigated the consequences of signaling pathway activation by mediators of MIA using immunohistochemistry and Western Blotting. Single-cell RNA sequencing of more than 10,000 cells showed that dorsal forebrain organoids represent the diversity of cell types in the developing human neocortex. While mediators of MIA do not significantly affect cellular composition of organoids, they induce differential gene expression in them. Currently, we are characterizing differentially expressed genes in distinct cell types. Taken together, we have established a human *in vitro* model system of MIA that allows us to untangle cellular and molecular changes at an unprecedented resolution.

Characterizing the human microglia response to pathological alpha synuclein species using induced pluripotent stem cells

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Microglia are multifunctional glial cells representing the resident macrophage population in the central nervous system (CNS). Responding to a variety of disease associated stimuli, microglia can get activated and initiate an inflammatory response in the CNS. Several studies have shown direct evidence for the presence of a microglia-mediated inflammatory response in neurodegenerative diseases including Parkinson's disease (PD). A pathological hallmark of PD is the presence of high molecular weight α-synuclein protein species (HMW α-Syn) in intracytoplasmic and intraneuritic aggregates. Several studies point towards pro-inflammatory properties of HMW α-Syn species. As past studies on microglial function in health and disease mainly relied on rodent models, the investigation of microglial function in a human system is urgently needed. We utilized an optimized protocol for the generation of induced microglia-like cells (iMGL) from human induced pluripotent stem cells (iPSCs) to investigate the human microglial response to different pathological species of α-Syn.

Assessing the functional role of niche astrocytes in regulation of adult hippocampal neurogenesis

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Astrocytes are one of the most abundant cell types in the mammalian brain and play increasingly appreciated roles in supporting brain development and function. Astrocytes are major part of the adult hippocampal stem cell niche, and have been shown to control several steps of neurogenesis, from neural stem and precursor cells (NSPC) proliferation to differentiation and maturation of newborn neurons. Recent work proved astrocyte heterogeneity among different brain regions and suggested that astrocytes contribute region-specifically to brain homeostasis and neural plasticity. Interestingly, our lab identified intra-regional astrocyte diversity in the adult dentate gyrus (DG) of the hippocampus. Specifically, three major astrocyte subtypes can be distinguished by their morphology and sub-localization to specific DG structures. These astrocytes exhibit subtype-specific molecular properties and can be discriminated and prospectively isolated based on molecular marker gene expressions. We hypothesize that these morphological, positional and molecular differences account for different functional properties during the process of adult neurogenesis. To test this, one of my first goal is to establish an *in vitro* system to evaluate if NSPCs proliferation capacity as well as differentiation and maturation of progeny is affected by co-cultures with different astrocyte subtypes. Furthermore, this system allows to test factors that are differentially expressed by distinct astrocyte subtype for their function in the neurogenic process. These findings may help us to better understand the role of astrocyte in neurogenesis and adult hippocampal plasticity.

Autophagy is a cell-intrinsic driver of neural stem cell quiescence in hippocampal dentate gyrus development

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Neurogenesis in the adult mammalian brain relies on the life-long persistence of quiescent neural stem cell (NSC) reservoirs. Little is known about the mechanisms that lead to the initial establishment of NSC quiescence during development. We here show that the autophagy machinery accumulates in quiescent NSCs and that pharmacological blockade of autophagy disrupts quiescence. We further demonstrate that autophagy is cell-intrinsically required to establish radial glia-like NSC quiescence during hippocampal development.

Using patient iPSC-derived neurons to elucidate the role of spastin in store-operated calcium entry

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Pathogenic variants in SPAST, the gene coding for spastin, are the single most common cause of hereditary spastic paraplegia (HSP), a progressive motor neuron disorder affecting mainly the axons of corticospinal motor neurons that is characterized by progressive spasticity of the lower limbs. Spastin is a microtubule-severing protein that in addition contributes to the ER-morphogenesis. How spastin dysregulation leads to axonal degeneration is still unclear. Increasing evidence shows that the endoplasmic reticulum (ER) and its interplay with the microtubule network is crucial for effective store-operated calcium entry (SOCE), a cellular process which is triggered by ER-Ca2+ store depletion, and requires reshaping of ER and of the ER-resident protein STIM1. Since spastin influences both microtubule network and endoplasmic reticulum structure, we hypothesized that spastin is necessary for the regulation of Ca2+ homeostasis via store-operated calcium entry. Here, we show that dysregulation of spastin alters the endoplasmic reticulum structure, alters the transport of STIM1, and has an impact on the Ca2+ regulation via SOCE, which is compromised in neurons derived from the induced pluripotent stem cells (iPSCs) of patients with pathogenic mutations in SPAST. Genome editing using CRISPR/Cas9 technology to correct the pathogenic variants in spastin, successfully restored spastin expression levels, Ca2+ regulation, and axonal integrity. Our results show that spastin is a key component in the regulation of Ca2+ homeostasis via SOCE and implicates SOCE dysregulation as a pathogenic mechanism in spastin-linked motor neuron disease

Impact of activin signalling on hippocampal GABAergic inhibition and its effects in neuropsychiatric disorders

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Adolescence is a critical period associated with behavioural and emotional changes. Aversive challenges like chronic stress during this period may increase susceptibility to neuropsychiatric disorders later in life. However, the pathophysiological mechanisms rendering the adult brain susceptible remain largely unknown. Studies have indicated an increase in GABAA receptors during this late developmental stage. Our previous work showed that activin, a member of the TGF-β family, modulates hippocampal GABAergic inhibition and impacts anxiety-like behaviour, using transgenic mice expressing dominant-negative activin receptor IB (dnActRIB). To compare the neuronal circuitry during adolescence (P30-45) and in adulthood (P90-P120), we first systemically examined GABAergic inhibition onto granule cells in dentate gyrus, a hippocampal sub-region closely linked to the antidepressant effect of activin signalling. Whole-cell voltage-clamp recordings from hippocampal slices showed a significant increase in GABAergic inhibitory postsynaptic currents (IPSCs) in granule cells from adolescent mice, compared to that from adult slices. Interestingly, acutely applied corticosterone produced mixed responses of IPSCs in adult dorsal slices, but a uniform suppression in ventral ones, indicating diverse impacts of stress hormone along hippocampal longitudinal axis. To elucidate the mechanisms involved in adolescent stress and its effects on adulthood, we then established a behaviour model by administering corticosterone during adolescence (P30-P45). Depression-like phenotype in adulthood was manifested in forced swim test with higher immobility in treated wild type, but not dnActRIB, mice. Preliminary data from hippocampal granule cells from stressed mice indicate a tendency of decrease in IPSC frequency, suggesting the long-lasting impact of adolescent stress on hippocampal synaptic transmission.

Modeling Neuro-Immune Interactions under Physiological Conditions: A novel Organoid-based Approach for Studying Human Environment-dependent Microglia Phenotypes

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Microglia are a specialized population of brain-resident macrophages that play a central role in brain development. However, until now, the ability to model the interactions between the human brain environment and microglia has been severely limited. To overcome these limitations, we have developed a novel approach that leverages recent advances in stem cell biology to mimic the invasion of erythromyeloid progenitors into cortical brain organoids and capitalizes on our ability to graft these units into a rodent host for vascularization. The integrated microglial cells (hMG) survive for extended periods of time, express microglia-specific markers and populate the human organoid graft. Furthermore, hMG show morphological features indicative of a resting and surveillance state and assume transcriptomic signatures that closely resemble their in vivo counterparts. Taken together, the system developed here will provide a platform for studying functional human brain-microglia interactions under physiological conditions and over extended periods of time, a critical next step for modeling human brain environment-dependent microglia phenotypes in health and disease.

The regulatory network of the cranial neural crest and molecular causes of orofacial clefts

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Orofacial clefts are the most common craniofacial birth defects. Although several gene mutations and inactivations have been identified over the years as causes of some orofacial clefts, the genetic origin of many other cases is still at large. We concentrate on four transcription factors and three chromatin remodelers whose inactivation in the cranial neural crest-derived mesenchyme leads to orofacial clefting in the mouse. By analysing their function and interactions in genome-edited cell systems and genetically altered mice, we will reconstruct the gene regulatory network that is required for orofacial development and palatogenesis. Our findings will identify genes and mechanisms involved in orofacial clefts. They may improve genetic counselling and pave the way to future therapeutic strategies.

In this study, we selectively knocked out the subunits Ep400, Tip60 and BRG1 of chromatin remodeling complexes as well as the transcription factors Sox5, Sox9, Sox11 and Tfap2a in the murine cranial neural crest cell line O9-1. To analyse transcriptomic alterations, RNA sequencing was performed and provided a broad dataset for extensive bioinformatical analyses and comparisons. The curated lists of up- and downregulated genes were used to perform gene ontology (GO, DAVID/GORILLA combined with ReViGo) and gene set enrichment (GSE, Broad Institute) analyses to determine pathways and biological processes, in which these factors play a role, such as proliferation, survival, migration or differentiation processes. These lists will also be compared with each other to determine common target genes and classify the exact effect of the various regulators on target genes as similar or antagonistic to determine the functional relationships between transcription factors and chromatin re-modelers. We will also compare our lists of up- and downregulated genes with lists of genes known to be relevant for palatogenesis and linked to orofacial clefting. These include lists of genes whose inactivation is known to cause syndromic CL/P or CPO or lists of genes that have been associated by genome wide association or linkage studies with a higher risk of non-syndromic CL/P or CPO. From these studies we expect to obtain a much clearer picture of the regulatory interactions and key effector pathways in the CNC-derived mesenchyme that are disturbed in CL/P or CPO.

Influence of promyelinogenic molecules on MAPK-mediated signal transduction and metabolic profile during oligodendroglial myelination.

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Oligodendrocytes, the myelinating cells of the central nervous system (CNS), concentrically wrap axons with multi-lamellar sheets of their plasma membrane both to enable rapid saltatory conduction of action potentials and to provide nutritional support for axons. In CNS diseases like multiple sclerosis (MS) and multiple system atrophy (MSA) loss of myelin sheaths is an important neuropathological hallmark. Since intrinsic remyelination is limited and often fails to restore functional myelin, the exploration of remyelinating strategies is crucial. Several antimuscarinics and thyromimetics have shown promyelinogenic effects in in vitro high-throughput screens and murine disease models. The underlying pathways driving myelination, however, remain poorly understood. The mitogen-activated protein kinase (MAPK) pathway, especially the extracellular signal-related kinases (ERK) 1 and 2, have been implicated in modulating oligodendroglial myelination. Here, we first examined the link between MAPK-mediated myelination and different antimuscarinics to gain more insights into the underlying molecular mechanisms using rodent-derived primary OPCs. We observed a synergistic effect of antimuscarinic compounds, but not thyromimetics combined with the treatment of an ERK inhibitor. We furthermore investigated the metabolic profiles of oligodendrocytes upon different treatments, which revealed that promyelinogenic compounds regulate glutamate, glycerophospholipid, and sphingolipid pathways differentially in primary rodent-derived OPCs. We next examined the rescue effect of these molecules in the context of MSA using OPCs derived from mice overexpressing human α-synuclein specifically in oligodendrocytes. Interestingly, thyromimetics were indeed able to rescue α-synuclein-mediated demyelination. Overall, by investigating compound-driven mechanisms for myelination, this study contributes to the discovery of new therapeutic approaches for demyelinating diseases.

C-Terminal binding protein 1 (CtBP1) controls proliferation and cell-fate determination during adult neurogenesis

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Neural stem cells (NSCs) are multipotent cells that can self-renew and differentiate into specialized cell types such as neurons and glia. The transition of NSCs to their mature phenotypes require complex reconfiguration of gene expressional profiles that occur at multiple cell-fate decision checkpoints. Transcriptional regulator, CtBP1, was recently implicated in mammalian neurodevelopment. Specifically, a patient cohort with a missense mutation in *CtBP1* was reported showing intellectual disability, ataxia and hypotonia. However, the putative function of CtBP1 in the context of neurodevelopment is underexplored. Herein, we studied functions of CtBP1 in adult neurogenesis using constitutive mouse model. We characterized adult hippocampal neurogenesis in CtBP1-knockout mice model immunohistochemically. Our data revealed that adult *CtBP1*$^{-/-}$ mice generate fewer immature and mature neurons *in vivo* than their wild-type counterparts. Next using BrdU pulse chase experiments, we found that CtBP1 ablation reduced the number of newly generated cells indicating its crucial role in maintenance of NSC proliferation pool. Additionally, we also generated *in-vitro* neurosphere cultures of NSCs from the subventricular niche of adult CtBP1 WT and KO mice. NSCs isolated from adult *CtBP1*$^{-/-}$ mice exhibited decreased self-renewal and an impaired ability to generate neurospheres after the primary passage. RT-qPCR profiling of these CtBP1-deficient neurospheres confirmed a lower expression of proliferation-linked genes and neuronal genes, and increased expression of glial markers. Collectively, these experiments demonstrate that CtBP1 is required for proliferation, differentiation and the cell fate decision-making process of adult NSCs.

De novo DNA methylation controls neuronal maturation during adult hippocampal neurogenesis

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Adult neurogenesis enables the life-long addition of functional neurons to the hippocampus and is regulated by both cell-intrinsic molecular programs and behavioral activity. The de novo DNA methylation machinery is crucial for embryonic brain development, but its role during adult hippocampal neurogenesis has remained unknown. Here, we show that de novo DNA methylation is critical for the maturation and functional integration of adult-born hippocampal neurons. Bisulfite sequencing revealed that de novo DNA methyltransferases target neuronal enhancers and gene bodies during adult hippocampal neural stem cell differentiation to establish neuronal methylomes and facilitate transcriptional up-regulation of neuronal genes. Inducible deletion of both de novo DNA methyltransferases Dnmt3a and Dnmt3b in adult neural stem cells did not affect proliferation or fate specification but specifically impaired dendritic outgrowth and synaptogenesis of new-born neurons thereby hampering their functional maturation. Consequently, abolishing de novo DNA methylation modulated activation patterns in the hippocampal circuitry and caused specific deficits in hippocampus-dependent learning and memory. Our results demonstrate that proper establishment of neuronal methylomes during adult neurogenesis is fundamental for hippocampal function.

The serine-threonine Kinase Ndr2 participates in spatial memory formation through an interplay between of autophagy and protein-wide alterations in the ageing brain

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The serine-threonine kinase Ndr2 regulates dendritic arborization and integrin signaling. Previous work from our group showed that young Ndr2 KO mice display impaired spatial memory, LTP and spine formation, demonstrating that the kinase is critically involved in neural plasticity and memory. Moreover, Ndr2 has been strongly implicated in the regulation of autophagy in the adult brain. Given that that autophagy dysregulation is paramount for memory formation and proper neuronal autophagy prevents cognitive decline associated with ageing, and that Ndr2 regulates memory and plasticity, the question of whether it play a role in the cognitive decline associated to autophagy dysregulation in old age, still remains. We demonstrated that Old Ndr2 KO mice have decreased autophagic flux in the dorsal hippocampus (DH) but paradoxically display better performance in the Morris water maze and novel object location tasks. Conversely, pharmacological upregulation of autophagy proved to be detrimental to spatial memory. By mass spectrometry we observed that old Ndr2 KO mice have fewer age-related changes in the DH proteome and that alterations in pathways related to neuroinflammation, proteostasis, regulation of synapse translation, and synaptic transmission are prevented. The fact that Ndr2 deficiency prevents DH dependent memory decline even though autophagy is impaired, as well as changes in the proteome, suggest that Ndr2 participates in the age-related cognitive decline through an interplay between autophagy and plasticity-related cellular processes.