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Abstract Booklet

Neurodevelopment and Vulnerability of the Central Nervous System

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Poster Session 1 Abstracts

Physiological roles of Amyloid Beta and APP-derived fragments on neuronal transmission

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The β-amyloid protein (Aβ) has been extensively studied for its involvement in Alzheimer's disease. However, the physiological function of Aβ remains mostly unknown. Amongst different species, research focused on the predominant isoform Aβ42, suggesting its hormetic effect on neuronal transmission. Recent reports suggest an important role of N-terminal fragment covering aa 1-17 of Aβ in neuroplasticity. However, as systematically investigation of Aβ42 derived fragments in cellular context was not performed yet.

To close this gap we explore the pre-and postsynaptic effects of the physiological concentration of Aβ fragments in rat cortical and hippocampal neurons. We visualized the efficiency of synaptic vesicle recycling by means of uptake of anti-synaptotagmin-1 antibody (Stg1Ab) in living neurons and correlated these data with outcome of quantitative analysis of synaptic expression of number of presynaptic and postsynaptic proteins implied in neuroplasticity. Finally, we also performed electrophysiological recordings to monitor effect on neurotransmission.

We found that application of picomolar concentrations of synthetic Aβ42 and Aβ1-16 increased presynaptic efficacy via activation of α7 nicotinic acetylcholine receptors (α7-nAChRs) while application of Aβ17-42 had no effect on neurotransmitter release; it had an effect in patch clamp recordings suggesting its action on the postsynaptic terminal. We could show that Aβ peptides covering the N-termini fragments induce changes in synapsin phosphorylation at multiple sites leading to a reorganization of the synaptic vesicle pools pointing to a possible involvement of CDK5/CNB signaling.

Together, our data suggests that Aβ fragments have compartment-specific roles and that the modulation of their production could represent a powerful physiological mechanism for tuning of neurotransmission at specific synapses in vivo.

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Post-translational regulation of the neurogenic transcription factor Sox11 modulates neuronal morphogenesis

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SOX11 is a key transcription factor that belongs to the SoxC (SRY-related HMG-box C) family and it is critical in the regulation of embryonic and adult neurogenesis. Loss of SOXC in the neural stem cells of adult mice, abolishes their neuronal differentiation. In contrast prolonged Sox11 expression in the NSCs of adult mice causes delayed maturation and reduced morphological complexity. SOX11 has gathered additional attention following recent discoveries implicating it in various pathophysiologies during development (Coffin-Siris Syndrome), axonal regeneration (CNS& PNS) and cancer, however, the regulation of its transient activity has not been addressed yet. In this study we found through Mass Spectrometry that SOX11 has at least 10 phosphorylated Serine residues. Mutational approaches showed that 3 of them are important for SOX11's subcellular localization and transcriptional activity. Additionally, we could show that SOX11 is phosphorylated by PKA on S133. Phospho-mutants of S133 revealed a critical role for this phosphorylation in the establishment of neuronal subtype-specific dendritic morphology possibly due to its distinct potency to transactivate promoters of target genes. Future studies will further address the question of how SOX11's phosphorylation is regulated.

RAB18 modulates autophagosome formation and is functionally rescued by ATG9A

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Protein homeostasis is of vital importance for cellular development and function. In a functional RNAi-screen in C. elegans aiming to identify new components of the proteostasis network, we characterized RBG1/RAB3GAP1 and RBG2/RAB3GAP2 as novel positive modulators of macroautophagy. Subsequently, we uncovered that the RAB GTPase RAB18 is the downstream effector of RAB3GAP1/2's autophagy activity and that these proteins support the acquisition of lipids from lipid droplets (LDs) for autophagosome formation.

Loss of function mutations in genes coding for RAB3GAP1/2 and RAB18 are linked to Warburg Micro syndrome (WARBM), a human neurodevelopmental disorder. Interestingly, the CRISPR/CAS9-mediated knockout of RAB18 and RAB3GAP1 in HeLa cells resulted in a noticeable LD phenotype, strikingly resembling the key cellular alteration in WARBM patient fibroblasts. Detailed analyses revealed that LDs in knockout cells are increased in size, cluster at the perinuclear region, and are blocked in consumption upon starvation, clearly indicating a disturbed LD-turnover. Interestingly, while the transient knockdown of RAB18 and RAB3GAP1 reduces autophagic flux, basal autophagic activity remains unchanged after their stable knockout. However, autophagy induction fails under these conditions, pointing towards a compensatory mechanism to maintain basal autophagy. Indeed, subsequent studies showed that the autophagic activity of ATG9A is increased in RAB18 knockout cells through the SRC kinase-dependent phosphorylation of ATG9A at Tyr8, which directs the transmembrane protein to autophagosome formation, thus, maintaining basal autophagy and compensating RAB18 absence. This illustrates the importance of RAB18 and LDs for macroautophagy and presents novel insights into cellular alterations that might be relevant for WARBM pathogenesis.

Learning deficits in rats overexpressing the dopamine transporter

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With its capacity to modulate motor control and motivational as well as cognitive functions dopamine is implicated in numerous neuropsychiatric diseases. Genetic predispositions and environmental triggers together shape the extend of behavioral alteration and subsequent disease status. The present study investigated whether an imbalance in dopamine homeostasis as evident in the dopamine overexpressing rat model (DAT-tg), results in learning and memory deficits associated with changes in adult hippocampal neurogenesis. Adult DAT-tg and control rats were subjected to the Morris water maze, the radial arm maze and a discrimination reversal paradigm and newly generated neurons in hippocampal circuitry were investigated post mortem. DAT-tg rats were found to exhibit a striking inability to acquire information and deploy spatial search strategies. At the same time, reduced integration of adult-born neurons in hippocampal circuitry was observed, which together with changes in striatal dopamine signaling might explain behavioral deficits.

EGFL7 - a neurovascular regulator of NSCs governing olfactory perception and behaviour

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Adult neural stem cells reside in a specialized niche in the subventricular zone (SVZ). Throughout life they give rise to adult-born neurons in the olfactory bulb (OB), thus contributing to neural plasticity and pattern discrimination. Here, we show that the neurovascular protein EGFL7 is secreted by endothelial cells and neural stem cells (NSCs) of the SVZ to shape the vascular stem-cell niche. Thus, ectopic expression of EGFL7 pushed aNSCs towards differentiation into TAPs, NBs and eventually interneurons. Conversely, loss of EGFL7 caused an accumulation of aNSCs within the SVZ and a smaller proportion of them entered the neuronal differentiation pathway. Consequently, less interneurons were formed in the OB of adult EGFL7_/_ mice. This indicates that EGFL7 is necessary for neuronal differentiation of NSCs but also affects their activity state.

To define whether or not the regulation of NSCs/NPCs by EGFL7 is of physiological relevance for the adult brain, the amount of adult-born neurons in the OB was determined and was found to be reduced in EGFL7_/_ but increased in AdEGFL7- infected mice. Decreased numbers of neurons in the OB of EGFL7_/_ mice increased the threshold for odorant detection in olfactory behaviours and reduced olfactory discrimination, which is in agreement with previous studies on the impact of mitral cell activity on mouse behaviour and the role of adult-born neurons in this context.

Collectively, this work identifies EGFL7 as a neurovascular regulator of NSCs, governing olfactory perception and behaviour.

Machine Learning support for Cyber-Physical Model-Driven Architecture for Robotic Applications

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Today, programming robots is a complex, time-consuming and expensive task. Wandelbots develops a product (Wandelbox) for end-user programming of self-adaptive robotic applications based on smart clothing. Regular pieces of clothes are equipped with sensors and actuators to track human motion. Based on the sensed information, robots can be controlled simultaneously. This principle enables people to show a robot a certain task by example. The robot motion is recorded and can be used to derive a robot control model, which can be used to generate platform-independent or platform-specific code. The overall process forms a domain specific Cyber-Physical Model-Driven Architecture (CPS-MDA).

The aim of my work is to design and implement a flexible approach to include machine learning techniques in the existing teaching infrastructure. The central idea is to use findings from manual mappings of continuous motion data to abstract processes to automatically generate a proposal for such an abstract process. Because the Wandelbox shall operate without the need for manual engineering work, a domain specific solution has to be developed. Furthermore, current implementations for machine learning integrations do not provide an infrastructure to change features and combine or exchange concrete implementations at runtime. Therefore, an approach and an architecture have to be designed, that enables runtime selection, extracting and mapping of features as well as the exchange of machine learning strategies at runtime. This requires the identification, specification of concrete domain constraints, potential features, extraction/mapping algorithms as well as machine learning implementations.

Disease modeling of rare neurodevelopmental diseases using human stem cell based models: improving analysis of neural differentiation by flow cytometry

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Neural differentiation of human pluripotent stem cells (hPSC) offers the possibility to create human based models for rare neurodevelopmental diseases. Coffin-Siris-Syndrome (CSS) is a rare genetic disorder and is clinically characterized by developmental delay or intellectual disability, microcephaly, and hypoplastic fifth distal phalanges. We generated neural rosettes, neuronal precursor cells (NPC) and neurons from human embryonic stem cells by applying a multi-step embryoid body protocol. These neural cells offer the possibility to study different stages of neurodevelopment. The hypothesis that was tested is that mutations in genes involved in CSS lead to stage-dependent modulations of neural subtypes during neural differentiation. To test this hypothesis, we established flow cytometry (FC) for high throughput, multiparameter analysis for single cells to quantify subpopulations in different stages of development. We optimized and evaluated following marker: CD24, a neuronal marker which is transiently expressed during human brain development. The neural stem cell marker CD133 is highly expressed in control NPCs. Together with neural crest marker CD271 we determined and quantify the subsets of central- and peripheral neural stem cells at the neural rosette stage. Following the neural differentiation paradigm, we got more specific insights at the neuronal precursor stage by adding neural marker CD56 (NCAM1) and CD15 in our analysis to identify cells with neuronal fate. Beside the NPC, we typically generate a subpopulation of astrocyte restricted precursors, which are identifiable by their expression of CD184 (CXCR4) and CD44 (HCAM). The heterogeneity of extra cellular marker expression on differentiated hPSC could give relevant insights in mechanisms of neurodevelopmental diseases.

An Increase in Neural Stem Cells and Olfactory Bulb Neurogenesis Improves Odor Discrimination

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The subventricular zone is the main source of neural stem cells (NSCs) in the mammalian brain and current strategies aim to manipulate them as a promising approach towards therapy. Our group has shown that the expansion of endogenous NSCs can be controlled by shortening the G1 phase of their cell cycle upon overexpression of Cdk4/cyclin D1 (4D). We generated a transgenic mouse that allows the temporal and reversible control of 4D overexpression. We found that switching on 4D increases the population of NSCs while switching it off afterwards allows their physiological differentiation. As a consequence of the transient expansion of NSCs, the final neuronal output is increased. 4D-derived neurons integrate in the olfactory bulb circuit and are undistinguishable from physiologically generated ones. Interestingly, mice with increased neurogenesis showed an improvement in olfactory performance only when discriminating between very similar odors.

Acyl-ghrelin regulates methylation of key neurogenic gene promoters

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Epigenetic mechanisms act as key modulators of gene expression and serve as a vital link between the environment and our genes. Adult hippocampal neurogenesis (AHN) is the process of generating new fully mature and functional neurones from pools of neural stem cells, located in a region of the brain called the hippocampus. This process is important in learning and memory. Dysfunction of AHN has been implicated in many neurodegenerative diseases (such as Parkinson's and Alzheimer's) and neurological conditions (such as depression). AHN has been shown to be regulated by many intrinsic and extrinsic factors, of which epigenetic modulation is one of them.

Acyl-ghrelin (AG), the orexigenic gut peptide and growth hormone secretagogue, promotes AHN and enhances pattern separation memory in rats (Kent et al. 2015). Notably, the pro-neurogenic effect of calorie restriction is mediated by the AG receptor, GHSR (Hornsby et al. 2016). However, the specific mechanisms underpinning AG-mediated AHN are still not fully understood. Ma et al. (2009) reported that electro-convulsive therapy increased AHN via induction of Gadd45b expression, an immediate early gene involved in DNA damage and stress response. They showed that Gadd45b orchestrated de-methylation of some key neurogenic gene promoters, including Bdnf IX and FGF-1b, leading to an increase in gene expression and subsequent enhancement of neurogenesis.

Hippocampal microarray data from our lab identified increased Gadd45b mRNA expression in mice that received a chronic 7-day infusion of AG. RT-qPCR analysis of the hippocampal extracts confirmed this finding. We now show that acute AG treatment (1mg/kg) also increases Gadd45b protein expression in both the mouse and rat hippocampal dentate gyrus. Indeed, using confocal microscopy we also show that the AG receptor, GHSR, is co-expressed with Gadd45b in the granular cell layer of the dentate gyrus.

To determine whether the AG-mediated increase in Gadd45b resulted in the de-methylation of key neurogenic promoter regions we performed preliminary methylation (MeDIP) and hydroxymethylation (hMeDIP) analysis using neuronal cell culture system (SN4741 cell line). AG treatment induced significant demethylation of the gene promotors for FGF1b and BDNF IV ($P<0.01$ & $P<0.05$). hMeDIP also showed a significant reduction in hydroxymethylation in FGF1b following AG-treatment ($P<0.01$), but was increased in BDNF IV ($P<0.05$). RT-qPCR analysis further showed upregulation of these gene transcripts ($P<0.05$).

These data suggest that AG promotes Gadd45b activity in the dentate gyrus resulting in de-methylation of FGF1b and BDNF IV gene promoters and enhanced neurogenesis and learning. Further studies are underway to test this putative molecular mechanism and to determine whether AG regulation of adult brain plasticity is mediated by dynamic epigenetic change.

Circulating unacylated-ghrelin impairs hippocampal neurogenesis and memory in mice and is altered in human Parkinson's disease dementia

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New neurones are formed from neural stem/progenitor cells (NSPCs) in the adult dentate gyrus (DG) throughout life and contribute to spatial pattern separation memory. Factors that promote neurogenesis may attenuate age-related cognitive decline.

Calorie restriction (CR) has been shown to modulate the DG and improve cognitive function, albeit via unknown mechanisms. Previously, we showed that the stomach hormone, acyl-ghrelin (AG), which is elevated during CR, increases neurogenesis in the DG and enhances pattern-separation memory (Kent et al.2015). The ghrelin-receptor (GHSR) is expressed in mature DG neurones in close proximity to DG NSPCs of adult mice, suggesting a non-cell autonomous mechanism of action. We also show that CR enhances neurogenesis in WT but not in GHSR-ko mice, demonstrating that CR induces AHN in a GHSR-dependent manner (Hornsby et al.2016).

Here, to determine whether unacylated-ghrelin (UAG), the so-called inactive form of ghrelin, regulates neurogenesis, WT and ghrelin-O-acyl transferase null mice (GOAT-ko) - that lack circulating acyl-ghrelin - were treated with vehicle or UAG for 7-days. Surprisingly, UAG-treated WT mice had reduced proliferating (Ki67+) cells, (DCX+) neurones and newborn (BrdU+/DCX+) neurones. GOAT-ko mice had similar reductions in neurogenic markers and impairments in hippocampal-dependent memory that were restored by acyl-ghrelin treatment.

Finally, we show that circulating AG:UAG in Parkinson's disease dementia was significantly reduced compared to both age-matched healthy controls and a cognitively normal PD group.

These data identify a novel role for UAG in regulating hippocampal plasticity and memory, and suggest that AG:UAG may be a biomarker of dementia in humans.

Role of Hippo signalling in adult neural stem cell homeostasis

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Adult neural stem cells (aNSCs) residing in the subgranular zone (SGZ) of the dentate gyrus (DG) are finely regulated to precisely control the adult generation of newborn neurons. Quiescence is essential for long-term maintenance of aNSCs. Intrinsic factors, as well as extrinsic niche signals, orchestrate the balance between activation and quiescence of aNSCs but the mechanisms are still not completely understood.

Here we show that changes in Hippo signaling pathway affects the quiescence and activation of aNSCs in mice. We found that Yap1, one of the most important effector components in Hippo pathway, is specifically expressed in mouse aNSCs and astrocytes in SGZ, but not in other cell types such as neurons and oligodendrocytes. Moreover, levels of nuclear Yap1 significantly decrease in induced quiescent aNSCs. In vitro, overexpression of constitutively active Yap1 (5SA) induces the transition from quiescence to activated state of aNSCs. This effect is exerted through TEAD interaction, thereby, constitutively active version of Yap1 with a mutation in TEAD binding site (5SA/S94A) fails to activate quiescent aNSCs. In addition, pharmacological disruption of YAP1-TEAD complex decreases proliferation of aNSCs. These results indicate that Yap1-TEAD interaction is very important for aNSCs activation and proliferation. In vivo, overexpression of constitutively active Yap1 driven by hGFAP promoter, increases proliferation of aNSCs dramatically at 7 dpi in DG. Moreover, overexpression of Yap1 can also block differentiation of aNSCs and maintain them at progenitor stage (Sox2 positive) at 30 dpi. Collectively, we uncover a new mechanism which can regulate aNSCs development and exhibit the key role of Hippo signaling in aNSCs homeostasis.

Investigating the pathomechanisms in Borjeson-Forssman-Lehmann syndrome

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Borjeson-Forssman-Lehmann syndrome (BFLS) is a syndromic form of X-linked intellectual disability caused by mutations in PHF6. PHF6 contains two plant-homeodomain-like (PHD) domains known from chromatin-interacting proteins, localizes to the nucleus and interacts with the PAF1 transcription initiation complex and with the NuRD complex, a multifunctional epigenetic regulator. Apart from that, little is known about its function and its role in nervous system development so far. Therefore, we aim to further elucidate the pathomechanisms in BFLS.

In order to assess the effect of different mutations, we established wildtype and mutant constructs of PHF6 and transfected HEK-293 and SK-N-BE cells. Wildtype PHF6 was found to be ubiquitously located in the nucleus and particularly in the nucleolus. In contrast, mutant PHF6 was also located within the nucleus but forming aggregates, which might impair its chromatin binding capacities.

We utilized CRISPR/Cas9 to create PHF6 knockout lines in HEK-293 and SK-N-BE cells. Using retinoic and caffeic acid we subsequently differentiated PHF6 knockout SK-N-BE cells into a distinct neuronal phenotype. Of note, by immunofluorescence on PHF6 knockout SK-N-BE cells we observed upregulation of nucleolus marker Fibrillarin. This was confirmed by quantitative RT-PCR and Western Blot and might point to a so far unrecognized role of PHF6 in nucleolar processes.

Additionally, we performed transcriptome analyses on RNA from patient lymphocytes and from different PHF6 knockout cell lines. We selected several potential target genes from the differentially expressed genes and are currently validating results by qPCR. In addition, we are establishing ChIP-Seq analyses to further detect specific targets of PHF6.

Our study will gain further insight into the role of PHF6 in nervous system development and function and into the molecular mechanisms underlying BFLS.

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βB2-crystallin mutations alter parvalbumin-positive interneuron number and prepulse inhibition.

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Aims: To understand the function of βB2-crystallin (Crybb2) in the mammalian brain, where it is widely expressed. So far, Crybb2 is known mainly for its role as an ocular structural protein and is suggested to act as a calcium buffer.

Methods: Using behavioural phenotyping, immunohistochemistry and stereology, we examined the effect of three different Crybb2 mutations in mice. All three mutations are located in the C-terminal globular domain of the protein. We assessed the influence of these alterations on different behaviours, expression patterns of Crybb2 and expression of the calcium-binding protein parvalbumin in selected brain regions.

Results: Of all behaviours and brain regions assessed, only one behaviour and one brain region were affected by all three mutations: sensorimotor gating behaviour as measured by prepulse inhibition of the acoustic startle reflex and parvalbumin expression levels in the thalamic reticular nucleus (TRN). The direction of these effects were correlated and mutation-specific: two Crybb2 mutations led to an increase in prepulse inhibition and a concomitant increase in the population of parvalbumin-positive neurons in the TRN, whereas both features decreased in the third.

Conclusion: These findings suggest that the parvalbumin-positive GABAergic interneurons of the thalamic reticular nucleus are involved in the neuronal circuitry that modulates prepulse inhibition of the acoustic startle reflex. As changes in parvalbumin-expressing interneurons and in prepulse inhibition are endophenotypes associated with schizophrenia, the reproducible alteration of both by three different Crybb2 mutations implicates this gene in this neurodevelopmental disorder.

KV1.4 role in autoimmune and toxic related remyelination in multiple sclerosis models

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Remyelination, the innate process of repair after demyelination, remains a hopeful target for multiple sclerosis (MS) therapy. However, complete understanding of this mechanism is not yet clear and therapies related to neuroprotection and remyelination remain unavailable. Research has shown that oligodendrocyte precursor cells are recruited into demyelinated zones and are able to differentiate into myelinating oligodendrocytes (Ols) providing a new myelin layer. Understanding of Ols physiology during such process is key to develop remyelination enhancement therapies. Here we analyze the role of the shaker type potassium channel, sub-unit KV1.4 in MS animal models. Despite being a developmentally restricted channel it re-expresses in Ols during the experimental autoimmune encephalomyelitis (EAE) model, suggesting a role in remyelinating processes. Moreover, mice lacking the KV1.4 (Kv1.4-/-) gene resulted in a much milder phenotype than its WT counterparts under EAE. We isolated oligodendrocytes from KV1.4 -/- and WT mice and cultivate them in proliferating conditions for 2 and 7 days which showed a marked decreased in proliferative cells in KV1.4-/- mice (WT d2=41,3%; KV1.4-/- d2=8,9%, p=0,016)(WT d7=29%; KV1.4-/- d2=3,2%, p=0,057). To shed light on the significance of these results, we will employ the demyelinating toxin cuprizone which renders robust demyelination in the corpus callosum after 5 weeks in the mice diet. In contrast with the EAE model, cuprizone exposure allows the study of remyelination and oligodendrocyte behavior in an environment free of peripheral immune cells. This data suggests a role of KV1.4 in remyelination and we expect to elucidate further the impact of this potassium channel in MS models.

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Structural and functional analysis of sporadic PD patient-derived neurons

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Parkinson's disease (PD) is the second most common neurodegenerative disease and characterized by progressive loss of dopaminergic midbrain neurons and their striatal projections associated with severe motor symptoms. Dysfunction of synaptic signaling, impairment of axonal transport and degeneration of axons are common early pathologic events in animal models and induced pluripotent stem cell (iPSC)-derived neurons of familial PD. The vast majority of PD patients, however, suffer from sporadic PD. We performed a comprehensive structural and functional analysis of the neuritic compartment of iPSC-derived midbrain neurons from sporadic PD patients and controls (Ctrl). After three weeks of midbrain differentiation using small molecules neurons from both groups expressed tyrosine hydroxylase and other dopaminergic marker proteins. PD-derived neurons did not show relevant differentiation deficits compared to Ctrl-derived neurons, but significantly less neuritic complexity. Directionality, frequency and velocity of axonal transport of mitochondria did not differ between Ctrl- and PD-derived neurons. Axonal distribution of mitochondria was also comparable between Ctrl- and PD-derived neurons indicating proper functionality of axonal mitochondrial transport. Midbrain neurons developed synaptic connections identified by expression of presynaptic protein Shank2 and postsynaptic protein Bassoon. Furthermore, midbrain neurons showed spontaneous as well as evoked activity in calcium imaging after three weeks of differentiation. The number of spikes, the average spike amplitude and the number of bursts was comparable between Ctrl- und PD-derived lines. In contrast to midbrain neurons from genetic PD patients, there was no obvious cellular phenotype detected in neurons derived from sporadic PD patients indicating that additional non-cell autonomous factors contribute to the pathogenesis in PD.

Wnt-signaling in maturation of adult-generated hippocampal neurons

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New dentate granule cells are generated in the hippocampus throughout life. These adult-born neurons arise from neural stem cells and undergo a multistep developmental sequence.
Wnt-induced signaling has been shown to play a crucial role in several aspects of adult hippocampal neurogenesis.
Using reporter mouse lines for canonical Wnt-signaling activity we could show a biphasic activity of the canonical pathway throughout the development of hippocampal neurons. While downregulation of Wnt-activity in neurons expressing the immature marker doublecortin (DCX) appears to be necessary for its proper development, re-activation of Wnt-signaling at the time point of synaptic integration points towards its importance for maturation.
To investigate the function of canonical Wnt-signaling in maturation we generated a transgenic mouse line that allowed activation of canonical Wnt-signaling in immature neurons via the expression of a stabilized β-catenin mutant. Premature activation of canonical Wnt-signaling resulted in downregulation of DCX, enhanced expression of mature neuronal markers and enhanced dendritic refinement, indicating that canonical Wnt-signaling activity accelerated maturation.
With increasing age mice exhibit impaired maturation of adult-born hippocampal neurons. Intriguingly, adult-born neurons in the ageing hippocampus also display a decrease in canonical Wnt-signaling activity, suggesting that impaired Wnt-signaling may contribute to the age-associated maturation deficit. Indeed, expression of stabilized β-catenin promoted maturation of adult-born neurons in aged mice.
In summary, the data emphasize that tight regulation of canonical Wnt-activity plays a crucial role in maturation of adult born neurons and also suggest that the age-related deceleration of neurogenesis is partly mediated by a decrease in Wnt-signaling activity.

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rAAV-mediated Expansion of Neural Progenitors in Neurodegenerative Disease Models

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In the mammalian brain, adult neural stem cells (NSCs) are found in the subgranular zone (SGZ) of the hippocampal dentate gyrus and in the subventricular zone (SVZ) of the lateral ventricle walls. Upon neuronal loss, several studies have shown that NSCs in the SVZ can differentiate and migrate towards the injury site. Our lab has found that overexpression of the CDK4/Cyclin D1 complex (4D) leads to the expansion of the NSCs by a shortened G1 and results in an increase in neurons that functionally mature and are indistinguishable from physiologically generated neurons (Artegiani et al., 2011). Moreover, mice with increased neurogenesis displayed an increase in cognitive performance (Berdugo-Vega et al., in preparation). Thus, the 4D-driven NSC expansion is appealing for the treatment of neurodegenerative diseases. However, our method so far required the use of transgenic animals or integrating lentiviruses which makes it not applicable in a clinical context. Therefore, we decided to use recombinant Adeno-associated viruses (rAAVs) being used in clinical trials. We show that serotype r3.45 efficiently infects SVZ NSCs recapitulating the 4D-mediated phenotype. Currently, mouse models of neuronal loss such as a photothrombotic or Endothelin 1 stroke and Huntington's or Parkinson's disease in the future are being explored as a preclinical model towards therapy.

Oligodendroglia α-synucleinopathy triggers early and regional-specific myeloid immune response in multiple system atrophy

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Multiple system atrophy (MSA) is a fast progressing atypical parkinsonian disorder which is neuropathologically characterized by intraoligodendroglial α-synuclein inclusions. We previously showed that oligodendroglial α-synuclein accumulation leads to maturation failure of oligodendrocytes consequently driving neurodegeneration. Notably, a severe neuroinflammation is observed in MSA and its corresponding mouse models. Here, we demonstrate a tight link between myeloid immune response and oligodendroglial α-synucleinopathy. Investigation of human putaminal post-mortem brain tissue of MSA-P patients and healthy controls revealed a 4.5-fold increased number of myeloid cells in putaminal white matter striae in MSA accompanied by elevated α-synuclein inclusions in contrast to putaminal gray matter. In order to examine the temporal and regional myeloid phenotype, we analyzed a MSA mouse model at two different time points, a pre-symptomatic (P21) and symptomatic (P90) disease stage. Mirroring our findings in human post-mortem tissue, we detected a highly increased number of IBA1+ cells in the corpus callosum and the striatum of transgenic animals already present in a pre-symptomatic disease stage. Additionally, these IBA1+ cells showed an elevated phagocytic activity and increased proliferation in white matter regions. RNA sequencing of α-synuclein-overexpressing primary oligodendrocytes revealed an upregulation of pro-inflammatory cytokines important for chemotactic attraction and proliferation of myeloid cells such as Ccl2, Cxcl10 and Csf1. Corresponding to the in vitro findings, an increased Ccl2 and Cxcl10 expression was identified in the corpus callosum and the striatum of MBP29-hα-syn mice compared to controls. Taken together these findings suggest that α-synuclein bearing oligodendrocytes.

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A novel environment-evoked transcriptional signature predicts reactivity in single dentate granule neurons

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Activity-induced remodeling of neuronal circuits is critical for memory formation. This process relies in part on transcription, but neither the rate of activity nor baseline transcription is equal across neuronal cell types. In this study, we isolated mouse hippocampal populations with different activity levels and used single nucleus RNA-seq to compare their transcriptional responses to activation. We found that, one hour after novel environment exposure, sparsely active dentate granule (DG) neurons had a much stronger transcriptional response compared to more highly active CA1 pyramidal cells and vasoactive intestinal polypeptide (VIP) interneurons. Activity continued to impact transcription in DG neurons up to five hours, with increased heterogeneity. By re-exposing the mice to the same environment, we identified a unique transcriptional signature that selects DG neurons for reactivation upon re-exposure to the same environment. These results link transcriptional heterogeneity to functional heterogeneity and identify a transcriptional correlate of memory encoding in individual DG neurons.

Sox11 – a novel activity-regulated gene with dentate gyrus-specific expression

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Neuronal activity initiates transcriptional programs that shape long-term changes in plasticity. Although neuron subtypes differ in their plasticity response, most activity-dependent transcription factors are broadly expressed across different neuron subtypes and brain regions. Thus, how regional and neuronal subtype-specific plasticity are established on the transcriptional level remains poorly understood. Here, we report that the developmental transcription factor SOX11 is induced in mature neurons upon hippocampal circuit activity and that this activity-dependent expression occurs exclusively in the dentate gyrus (DG) of the hippocampus. In addition, we show that SOX11 can modify intrinsic excitability of DG granule cell neurons most likely by altering membrane conductance. We propose that SOX11 is a DG-specific activity-dependent gene and might play a role in fine tuning regional plasticity in the hippocampal circuit.

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Impact of SOX11 on human early neurodevelopment

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The SoxC family of transcription factors controls a number of key processes in murine CNS development including neuronal fate determination, migration, neurite growth, neuronal survival and synapse development. While the function of SoxC in mice has been well characterized, the role of SoxC members in human neurodevelopment remains largely unknown. Heterozygous missense mutations or deletions in SOX11 have recently been identified as the underlying genetic cause in children with a Coffin-Siris Syndrome (CSS) like syndrome. Hallmarks of CSS include developmental disability, skeletal abnormalities, and characteristic facial features. The involvement of multiple organ systems in CSS, indicate that SOX11 fulfills critical functions in the development of different germ layers. Here, we investigate the function of SOX11 in human development using human embryonic stem cells (hESCs) as a model system. Homozygous SOX11-deficient hESCs lines with a SOX11 frameshift mutation were generated using CRISPR/Cas9 genome editing. In recent years different approaches have been optimized to study the development of human brain: 2D hPCS (human pluripotent stem cells) neural differentiation models, which mimics the environment that produces the neuroectoderm though embryoid body formation, and hPCS-derived 3D brain organoids. In both models we found that Sox11 deficiency was associated with decreased expression of Nestin, Pax6 and Sox1 upon induction of neural differentiation. Moreover, we observed that Sox 11 deficiency resulted in strong impairment in the generation of neurons. Collectively, our data point at an essential role for Sox11 during early stages of human neural development.

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Acid Sphingomyelinase is an Important Regulator of TRPC6-mediated Calciuminflux and Signaling

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Major depression is a life threatening disease affecting people worldwide. Currently, the enzyme acid sphingomyelinase (ASM, EC 3.1.4.12), that cleaves sphingomyelin to the bioactive lipid messenger ceramide, is being investigated as a potential target for antidepressant action. ASM and ceramide play a crucial role in distinct physiological and pathophysiological functions like lysosomal storage, neurogenesis, neuronal maturation and survival, and modulate membrane properties. Interestingly, some antidepressants, called functional inhibitors of ASM (FIASMA), indirectly inhibit ASM-activity. St. John's Wort is an herbal remedy in the treatment of depression disorders. Its active ingredient hyperforin activates plasma membrane bound transient-receptor-potential-canonical-6 (TRPC6) cation channels, which leads to Ca^{2+}-influx and phosphorylation of CREB resulting in beneficial neuronal modulations. In our study, we aim at investigating the interplay between the ASM/ceramide system and the TRPC6 channel function.

Hyperforin-induced Ca^{2+}-influx was examined in FIASMA-treated cell lines, mouse ASM-knockout synaptosomes and ASM-knockout cortical neurons using Fura-2 imaging. Hyperforin-induced CREB-phosphorylation was investigated in rat cortical neurons using quantitative immunostaining. In PC12 cell line, inhibition of ASM-activity by application of the FIASMA fluoxetine led to an impaired hyperforin-induced Ca^{2+}-influx. A decline in Ca^{2+}-influx was also revealed ex vivo in synaptosomes isolated from ASM-knockout mouse and by live-imaging of ASM-knockout cortical neurons. In rat glutamatergic cortical neurons, hyperforin induced significant CREB phosphorylation, whereas pretreatment with the ASM-inhibitor ARC39 diminished this effect.

Our data reveal the importance of balanced ASM-activity for TRPC6 function. Further studies shall investigate how the ASM/ceramide system and TRPC6 signaling interact in the pathogenesis of major depression.

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Identification of further individuals with de novo CTCF mutations refines the phenotypic spectrum, and altered dosage causes locomotor defects in Drosophila

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The architectural protein CCCTC-binding factor CTCF is essential for establishing and maintaining the three-dimensional organization of eukaryotic genomes. CTCF is involved in virtually all chromatin regulating processes including enhancer-promotor interactions and chromatin loop formation.
Recently, we identified de novo mutations in CTCF in four patients with a surprisingly mild phenotype of variable intellectual disability, mild microcephaly, and behavioral anomalies. Therefore we aimed at expanding the phenotypic spectrum associated with CTCF mutations and identified 15 further individuals with de novo aberrations in CTCF. In a total of now 19 cases we identified eight missense and seven truncating mutations and one single exon deletion, all but two located in one of the 12 zinc-finger domains, and three larger deletions. The phenotype is highly variable with mild to severe intellectual disability (18/19), autistic features (10/19), microcephaly (9/19), short stature (7/19), congenital heart defects (8/19) and palatal anomalies (7/19).
Apart from two conditional knockout mouse models with brain malformations and early lethality or learning deficits, little is known about the role of CTCF in neurodevelopment. Therefore, we utilized Drosophila melanogaster as a model to explore the role of CTCF in CNS development. Similar to observations in mice, ubiquitous depletion of Ctcf is embryonic lethal in Drosophila. We hence utilized the UAS/GAL4 system to induce tissue specific knockdown or overexpression of Ctcf.
Using the negative gravitaxis assay to examine gross neurological function, we found a highly significant impairment of locomotor behavior in flies with Ctcf knockdown in neurons and motoneurons and in flies with overexpression of Ctcf in glia cells and motoneurons.
We further delineate the mutational and clinical spectrum of CTCF-associated developmental phenotypes. Our findings of neurological anomalies upon manipulation of Ctcf dosage emphasize the role of Ctcf in nervous system development and function.

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Fibroblasts and reprogrammed neural progenitor cells of depressed patients show altered mitochondrial function

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Aims

We try to identify molecular pathomechanisms involved in Major Depressive Disorder (MDD) by characterization of mitochondrial function in cells derived from depressed patients.

Methods

Skin biopsies from 8 MDD patients as well as 8 gender- and age-matched healthy controls were obtained to grow human skin fibroblasts in cell culture. In a second step, fibroblasts were reprogrammed to induced pluripotent stem cells (iPSCs) using the Yamanaka protocol. After quality control, selected iPSC lines were differentiated into neural progenitor cells (NPCs). Patient-specific and control lines of both fibroblasts and NPCs were subjected to characterization of mitochondrial function. We used cationic dyes and fluorescence microscopy to analyze mitochondrial membrane potential (MMP) and investigated oxidative phosphorylation (oxygen consumption) and ATP synthesis by respirometry and luminometric ATP assays.

Results

MMP was significantly lower (less hyperpolarized) in MDD fibroblasts compared to healthy controls. The same tendency was observed in the NPCs derived from MDD patients. Respirometry experiments revealed a tendency of lower metabolic parameters in MDD fibroblasts for basal and maximal respiration as well as for spare capacity. ATP content in MDD fibroblasts and NPCs is lower compared to healthy controls.

Conclusions

Our findings are in line with the hypothesis that mitochondrial dysfunction in conjunction with a reduced metabolic capacity and energy (ATP) provision is involved in the etiology and pathophysiology of Major Depressive Disorder. More experiments are needed to investigate the cellular resilience to metabolic or hormonal stress.

A systems-level analysis reveals multiple concurrent layers of miR-124 regulation during human neurogenesis

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Non-coding RNAs regulate many biological processes including neurogenesis. The brain-enriched miR-124 is assigned as a key player of neuronal differentiation via its complex, but little understood, regulation of thousands of annotated targets. To systematically chart its regulatory functions, we used CRISPR/Cas9 gene editing to disrupt all six miR-124 alleles in human stem cells. Upon neuronal induction, miR-124-deleted cells underwent neurogenesis and became functional neurons, albeit with altered morphology and neurotransmitter specification. By RNA-induced-silencing-complex precipitation, we found that other miRNA species were upregulated in miR-124 knockout neurons. Furthermore, we identified 98 miR-124 targets of which some directly led to decreased viability. We performed advanced transcription-factor-network analysis and revealed indirect miR-124 effects on apoptosis and neuronal subtype differentiation. Our data emphasizes the need for combined experimental- and systems-level analyses to comprehensively disentangle and reveal miRNA functions, including their involvement in the neurogenesis of diverse neuronal cell types found in the human brain.

The Effect of IL4/STAT6 Pathway Activation in the Neural Progenitor Cells of Adult Mouse Hippocampus

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Alzheimer's disease is the most frequently occurring dementia as of 2018. During the course of the disease an abnormal accumulation of amyloid beta and tau proteins leads to a degeneration of neurons and synapses. The ongoing cell death coupled with inflammation in the absence of a regenerative capacity leads to an atrophy of the brain tissue and ultimately results in death of patients (Alzheimer's Association, 2018; Tincer, 2016). Harnessing the potential of the neural stem cells (NSCs) in a degenerative context with the aim to activate the proliferative-neurogenic cascade could be a potential therapeutic tool. Therefore, we aim to understand the molecular mechanisms behind the regenerative ability of zebrafish and consequently activate those cascades in our mouse model thus unlocking the proliferative and neurogenic potential of mouse NSCs.

As we have previously shown, the IL4/STAT6 pathway is activating the NSPCs in the zebrafish brain. The IL4 secretion upon Ab42 aggregation acts directly on the IL4 receptors of the Neural Stem and Progenitor Cells (NSPCs) leading to an increased proliferation and neurogenesis (Bhattarai, 2016).

In our recent study, IL4 seems to also play role in increased neurogenic potential of human NSPCs in the 3D culture system (Papadimitriou, 2018).

Interestingly in our mouse model, the IL4 receptor as well as the IL4 are not expressed in the NSCs of the hippocampus. Therefore, using a lentiviral system we have overexpressed the IL4 receptor in vivo and in vitro to assess its effects on the NSCs of the mouse hippocampus upon presence of IL4.

Influence of antimuscarinic molecules on MAPK-mediated signal transduction in 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 to enable rapid saltatory conduction of nerve impulses and provide nutritional support. In CNS diseases like multiple sclerosis (MS) loss of myelin sheaths is observed. In order to protect neurons from severe degenerative processes remyelination occurs driven by local proliferation and differentiation of oligodendrocyte precursor cells (OPCs). Since intrinsic remyelination is limited and fails to completely restore compact myelin the discovery of remyelinating strategies is crucial. Several antimuscarinic molecules showed promyelinogenic effects in in vitro high-throughput screenings and different MS mouse models. To better understand underlying mechanisms, we established a cell culture system of primary rat OPCs. After application of antimuscarinic molecules we detected increased myelin basic protein (MBP) gene expression using quantitative real-time PCR. However, the underlying pathways of this promyelinogenic effect remain poorly understood. It has been shown that the mitogen-activated protein kinase (MAPK) pathway, in particular the extracellular signal-related kinase (Erk) 1 and 2, significantly influences oligodendroglial myelination. Interestingly, oligodendroglial activation of Erk 1 and 2 increases myelin sheath thickness during development. In this project, we aim to examine the link between MAPK-mediated myelination and antimuscarinic molecules to gain more insights into molecular mechanisms driving myelination. Therefore, we plan to interfere with different MAPK dependent signals after application of promyelinogenic molecules. Additionally, validation of these promyelinogenic molecules will be analyzed in the context of neurodegenerative demyelinating diseases (e.g. multiple system atrophy).

Association of serum acid sphingomyelinase activity with alcohol dependence-related liver parameters

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Acid sphingomyelinase (ASM) catalyzes the hydrolysis of the lipid sphingomyelin into ceramide and thereby changes the local composition of the plasma membrane with effects on receptor-mediated signaling. Altered enzyme activities have been noted in a variety of common human diseases including alcohol dependence. However, the underlying mechanisms remain largely unresolved.

Blood samples were collected from early-abstinent alcohol-dependent in-patients (n[♂] = 113, n[♀] = 87) and from healthy control subjects (n[♂] = 133, n[♀] = 107) recruited for the Neurobiology of Alcoholism (NOAH) study during 2013-2015. We analyzed routine blood parameters provided by the central laboratory and ASM activity determined by hydrolysis of fluorescently labelled sphingomyelin.

We confirmed previous findings with increased ASM activity in alcohol-dependent male and female patients compared to healthy control subjects ($p<0.001$) and a decrease of ASM activity during alcohol withdrawal ($p=0.007$ for males). ASM activity correlated positively with liver enzymes and parameters of alcohol consumption (GOT, GPT, GGT, homocysteine, CDT, $p<0.001$) as well as myelosuppression (leukocytes, erythrocytes, thrombocytes, $p<0.030$) in male patients while fewer but similar correlations were found in female patients (GOT, GPT, GGT, CDT, and thrombocytes, $p\leq0.001$). Interestingly, the association of GGT with ASM activity was also found at physiological levels in healthy subjects (males $p=0.012$, females $p=0.001$).

We detected so far unreported associations of sphingomyelinase activity with markers of liver damage and myelosuppression. Further research should investigate whether there is a causal relationship or whether these parameters are part of a common pathway to gain insight into underlying mechanisms and develop clinical applications.

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Expelled and degraded: posttranslational control of a key neuronal fate determinant in SVZ adult neurogenesis

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Adult neurogenesis is regulated by stem cell niche-derived extrinsic factors and cell-intrinsic regulators, yet the mechanisms by which niche signals impinge on the activity of intrinsic neurogenic transcription factors remain poorly defined. Here, we report that the atypical TALE-homeodomain transcription factor MEIS2 is under dual posttranslational regulation in the subventricular zone (SVZ) / olfactory bulb neurogenic system. MEIS2 is an essential regulator of adult SVZ neurogenesis as it enables chromatin decompaction and effective transcription of neuron-specific genes by facilitating the assembly of a PBX1-PAX6-PARP1/ARTD1 containing complex at promoter-proximal regions of these genes. We here show that MEIS2 accumulates in the cell nucleus following down-regulation of EGFR signaling. Nuclear accumulation is modulated by methylation of MEIS2 on a conserved arginine, which lies in close proximity to nested binding sites for the nuclear export receptor CRM1 and the MEIS dimerization partner PBX1. Methylation impairs interaction with CRM1 without affecting PBX1 dimerization and thereby allows MEIS2 nuclear accumulation. In addition, MEIS2 is highly unstable in its non-nuclear form. MEIS2 protein stability inversely correlates with EGFR-activation and phosphorylation of MEIS2 on a serine / threonine motif within a predicted PEST sequence can serve as destruction signal leading to fragmentation and destabilization of MEIS2. Finally, we provide evidence that a protease, previously not implicated in SVZ neurogenesis, is responsible for MEIS2 degradation in SVZ-derived progenitor cells.

Our results reveal how MEIS2 integrates extrinsic signals to convert them into a neurogenic transcriptional program.

Heterogeneity amongst type I cells in the subgranular zone of the dentate gyrus

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The subgranular zone (SGZ) of the murine dentate gyrus harbors type I stem cells that continue to generate granule neurons during an animals life. Following a chronic in vivo imaging approach, we previously had monitored type I stem cells targeted by the Ascl1 promotor and observed a burst of neurogenic activity with subsequent depletion of the stem cell. This behavior was reminiscent of a developmental-like program but could not explain the proposed long-term self renewal capacity of stem cells. Therefore we used the Gli1 driver to target a different subset of type I cells. Indeed, the activation of Gli1 targeted type I cells is more spread throughout the observational period, compared to the Ascl1 targeted population which in majority gets activated in the first 20d. On a populational level this results in a constant generation of type II progenitor cells and neurons over an extended period which is different to the neurogenic burst observed in the Ascl1 driven population. However the average neuronal output per active type I cell is five neurons in both Gli1 and Ascl1 targeted cells. On a single cell level, activated Gli1 targeted type I cells showed extended quiescence periods between proliferative phases. To address the transcriptomic differences underlying the behavior of Gli1 and Ascl1 populations isolated type I cells short after recombination were analyzed by single cell sequencing. In conclusion, subsets of type I cells targeted by different driver lines exhibit differing dynamics and gene expression profiles and might serve for diverse demands in neurogenesis.

Patient-specific model of motor neuropathy in SPG11-linked motor neuron disease

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Mutations in SPG11 are the most frequent cause of autosomal-recessive complicated hereditary spastic paraplegia. The core symptom is progressive spasticity of the legs caused by degeneration of corticospinal motor neurons. Most patients also show degeneration of alpha motor neurons (aMN), leading to distal muscle wasting, weakness and dysphagia which impair quality of life and cause premature death. Still, there is no causal or disease modifying treatment for SPG11.

As a human model of aMN degeneration in SPG11, we have reprogrammed fibroblasts derived from SPG11 patients with neuropathy into induced pluripotent stem cells (iPSC). iPSC were differentiated into mature aMN, as shown by gene expression, protein expression, and electrophysiology. Proliferation of SPG11 aMN was unchanged. However, we delineate an axonal dysfunction phenotype in SPG11 aMN, characterized by reduced neurite growth and slowing of axonal transport. Ultrastructurally, SPG11 aMN exhibited accumulation of autophagosomes and autophagolysosomes, corresponding to previous studies in an SPG11 knockout mouse model and human non-neuronal cell models. Thus, iPSC derived aMN provide a valid cellular model of neuropathy and underlying autophagy deficits in SPG11 and will broaden mechanistic and therapeutic understanding of SPG11 related neurodegeneration.

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Using human iPSCs derived neurons to shed light on pathogenic mechanisms linked to SPG4

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Mutations in the SPG4 gene coding for spastin account for roughly 40% of all Hereditary Spastic Paraplegia (HSP) cases. Patients harboring SPG4 mutations progressively develop lower limb spasticity and paraplegia caused by length dependent degeneration of the corticospinal tracts.

Extensive research has focused on the microtubule severing activity of spastin, however, increasing evidence highlights the endoplasmic reticulum (ER) as a key player in the pathophysiology of HSPs. Of note, most of the research has been performed on cancer cell lines, but conversely, very little is known about the role of spastin in neurons, especially, in neuronal electrophysiology.

To study the relevance of spastin in the CNS we generated neurons from human induced pluripotent stem cells (hiPSCs), previously reprogrammed from SPG4 patients' fibroblasts. Using CRISPR/Cas9 technology we genome edit SPG4 mutations to generate isogenic controls. We used whole cell patch-clamp to analyze the electrophysiology of single iPSC derived neurons and analyzed the excitability and formation of networks in neuronal populations using a multielectrode array system. To study the ER on these neurons we used calcium imaging to examine the ability of the ER in maintaining calcium levels.

Our results indicate that spastin alters the ER morphology and plays a crucial role in the regulation of store operated calcium entry (SOCE) after calcium store depletion in mature iPSC derived neurons, opening a new door for a possible therapeutic target in the treatment of SPG4-HSP.

hiPSCs derived neurons are a powerful tool to study neuronal physiology and pathophysiology as well as the beginning and course of a disease.

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Ror2-signaling is required for local upregulation of GFD6 and activation of BMP signaling at the neural plate border

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The receptor tyrosine kinase Ror2 is one major Wnt receptor that activates β-Catenin independent signaling and plays a conserved role in the regulation of convergent extension movements and planar cell polarity in vertebrates. Mutations in the ror2 gene cause Recessive Robinow Syndrome in humans, a short-limbed dwarfism associated with cranio-facial malformations. Here we show that Ror2 is required for local upregulation of gdf6 at the neural plate border. Ror2 morphant embryos fail to upregulate neural plate border genes and show defects in the induction of neural crest cell fate. These embryos lack the spatially restricted activation of BMP signaling at the neural plate border at early neural stages, which is required for neural crest induction. Ror2-dependent planar cell polarity signaling is required in the dorso-lateral marginal zone during gastrulation indirectly to upregulate the BMP ligand GDF6 at the neural plate border and GDF6 is sufficient to rescue neural plate border specification in Ror2 morphant embryos. Thereby, Ror2 links Wnt/planar cell polarity signaling to BMP signaling in neural plate border specification and neural crest induction.

Long-term effects of an enriched environment on hippocampal adult neurogenesis and the behavior of mice

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The adult hippocampal neurogenesis is genetically controlled and can be enhanced by various behaviors, such as physical activity and living in an enriched environment (Kempermann et al, 1997; van Praag et al, 1999; Kempermann and Gage, 2002). Living in a complex, enriched environment can increase the survival of new neurons and partially compensate the age-related decrease of nerve cell formation (Kempermann et al, 1997; Kempermann et al, 2002; Freund et al, 2013).In addition, a complex environment even promotes the development of individual behavioral patterns. Freund et al. were able to demonstrate that inter-individual differences in exploration develop between C57BL/6 mice during husbandry in an enriched environment. These differences increase over time and correlate with adult neurogenesis. In order to further elucidate the relationship between individual behavioral differences and adult neurogenesis, I would like to investigate whether interrupting the enriched environment leads to a change in adult neurogenesis, that means whether the inter-individual differences in adult neurogenesis can be reversed. The results of my work will give information about how individual experience of an enriched environment have influences on behavior and brain plasticity and make it possible to provide behavioral guidance for cognitive-healthy aging. To investigate this I will use three different groups of experimental animals in my experiment. The first group of C57BL/6 mice is kept in an enriched environment for three month and then in standard cages for another three month. One control group is kept under standard conditions for six month and another control group in an enriched environment. After this period I will compare the different groups regarding behavior and nerve cell formation.

Functional heterogeneity and dynamic of astrocytes in the adult mouse hippocampus

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The brain works as a functional co-operation unit between neurons and glial cells, which differs in its physiological properties in distinct brain regions and developmental stages. Neuronal diversity has been extensively investigated. Recent works now suggest that also astrocytes are molecularly and functionally distinct, yet little is known about astrocyte heterogeneity. Using a genetic labeling strategy, we found that the adult hippocampal dentate gyrus is populated by morphologically distinct astrocytes that are localized to specific compartments. In contrast to the prevailing assumption that astrocytes are postmitotic in the non-injured adult brain, preliminary experiments revealed proliferation of non-radial astrocytes in the adult hippocampal niche. Even more surprising was the finding that morphologically distinct astrocytes show a differential proliferation response in the context of specific stimuli (voluntary exercise and ageing). This leads to the hypothesis that the dentate gyrus is composed of molecularly and functionally distinct astrocytes whose dynamics are critical modulators for hippocampal adaption to changing conditions. In order to assess structural astrocyte heterogeneity we carried out a detailed morphological analysis of distinct astrocyte subtypes and assessed their “connectome”, i.e. which other niche cells and structures are in direct contact to the astrocytes. Next, proliferation capacity and dynamics of distinct astrocyte subtypes was investigated under different physiological conditions. Furthermore, we investigated potential lineage relationships of astrocyte subtypes and radial glia-like neural stem cells by clonal analysis. Collectively, our study revealed structural heterogeneity and subtype-specific dynamics of hippocampal astrocytes in response to physiological stimuli.

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Cyclin D2 is required for on-site generation of adult neural stem cells during postnatal development of the dentate gyrus

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The dentate gyrus (DG) holds a specialized stem cell niche at the border between the granule cell layer and the hilus - the subgranular zone (SGZ) - that produces new neurons throughout life. Morphogenesis of the DG is complex and continues from around mid-gestation to early postnatal periods, ultimately leading to the formation of the SGZ during the second postnatal week in mice. Our previous studies revealed that the production of new neurons in the adult SGZ critically depends on cyclin D2 (D2). The neural progenitors expressing D2 are highly proliferative, unlike such expressing cyclin D1. Interestingly, we found only D2 expressed in adult radial glia-like stem cells (aRGC), indicating a prominent role of D2 for their proliferation and self-renewal. By using D2KO/Nestin-GFP double-transgenic mice we observed that the DG of adult D2KO mice is virtually devoid of radial NSCs and none of them is actively proliferating. Hence, we examined the role of D2 for the postnatal formation of the adult NSC pool (P0 to P28). Our studies reveal that early postnatal NSC niche development (hilar tertiary matrix) and the subsequent settlement of GFP-positive NSCs along the SGZ proceed also without functional D2, probably driven by D1 which is expressed by developmental NSCs. However, in D2KO mice the radial NSC pool fails to expand from P7 to P14, culminating in virtually complete loss of aRGCs at P28. Together, these results suggest that the aRGC pool is generated during a discrete critical period of postnatal DG development in a D2-dependent manner.

The synaptogenesis and synapse maintenance factor Bassoon: a putative factor for the differential sensitivity of photoreceptors to late-onset degeneration?

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Cone photoreceptors display particular sensitivity to degeneration as they rely on the integrity of the rod photoreceptor population for function and survival (Yu et al., 2004). The basis of the cone photoreceptor sensitivity remains unclear but may involve synaptic pathways and proteins. In mutant mice lacking functional Bassoon protein (Bsn^{mt}), we observed late-onset cone photoreceptor degeneration, while rod photoreceptors survived and reacted with structural and functional remodeling (Specht et al., 2007). The goal of this project is to shed light on the putative role of Bsn in conferring differential vulnerability to rod and cone photoreceptors.

As Bsn^{mt} mice still express a residual Bsn protein, which is diffusely distributed in photoreceptor synaptic terminals, we started to examine a mouse line with a full genetic deletion of Bsn (Bsn^{ko}). ERG recordings from Bsn^{ko} mice demonstrated a functional retinal phenotype - impaired photoreceptor synaptic transmission - that is comparable to Bsn^{mt} mice. To our surprise, however, we found no signs of cone photoreceptor degeneration so far. This raises the possibility that the Bsn remnant in Bsn^{mt} mice exerts a dominant negative effect on cone photoreceptor survival. We will perform comparative expression profiling for wild-type, Bsn^{mt}, and Bsn^{ko} photoreceptors in the search for putative dysregulated cellular pathways in Bsn^{mt} cone photoreceptors.

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Nfat/calcineurin signaling promotes oligodendrocyte differentiation and myelination by transcription factor network tuning

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Terminal differentiation of oligodendroglia depends on multiple classes of transcription factors. Among these are the bHLH protein Olig2, the SRY-box protein Sox10 and the homeobox protein Nkx2.2. Olig2 is already expressed in neural stem cells, Sox10 is expressed directly after lineage specification and expression of Nkx2.2 starts immediately before the differentiation process. Intriguingly, during the phase of oligodendroglial specification Olig2 and Nkx2.2 are expressed in different domains of the ventricular zone and cross-repress each other. We asked how expression of Nkx2.2 is achieved despite this cross-repression in later stages of oligodendroglial development. We identified enhancers of Nkx2.2 that are bound and activated by Sox10. Expectedly, Olig2 interfered with Sox10-dependent activation. Searching for factors that can overcome this Olig2-dependent inhibition, we found that Nfat transcription factors are expressed in oligodendroglia and that expression of Nfatc2 is dependent on Sox10. Nfat factors are activated by the Ca^{2+}-dependent phosphatase Calcineurin. Calcineurin gain-of-function can indeed overcome the cross-repression of Olig2 and Nkx2.2. Deletion of Calcineurin in oligodendrocyte precursor cells results in a drastic decrease of mature oligodendrocytes in vivo. Moreover, in primary oligodendroglial cultures the Calcineurin inhibitor FK506 interfered with terminal differentiation. We conclude that activation of Nfat transcription factors via Calcineurin is an important process to overcome cross-repression of Olig2 and Nkx2.2, allowing proper terminal differentiation of oligodendroglia.

Formation of toxic α-synuclein assemblies by glucosylceramide in human midbrain dopamine neurons

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Parkinson's disease (PD) is characterized by the aggregation of α-synuclein, resulting in the death of dopaminergic neurons within the substantia nigra of the midbrain. Over the past years, the number of genetic risk factors for PD connected to lysosomal proteins or pathways have increased steadily, clearly linking lysosomal dysfunction to PD development. One well-described genetic PD risk factor is the lysosomal hydrolase β-glucocerebrosidase (GCase), which degrades glucosylceramide (GluCer) within the lysosome. Although disrupted clearance of GluCer has been recently described to be involved in α-synuclein aggregation, the mechanisms of GluCer-induced α-synuclein aggregation are not completely understood.

By utilizing α-synuclein overexpressing cell lines and induced pluripotent stem cell (iPS)-derived dopaminergic midbrain neurons from healthy and PD donors, we document the presence of physiological α-synuclein conformers and tested their contribution to the aggregation process. Pathological α-synuclein assembly mainly occurred through the conversion of high molecular weight (HMW) physiological α-synuclein conformers into compact, assembly-state intermediates by the presence of GluCer. Reducing GluCer in PD patient neurons diminished pathology and restored physiological α-synuclein conformers that associated with synapses.

Our data suggests that GluCer contributes to pathogenic structural changes in α-synuclein promoting cell toxicity. Thus, GluCer-reducing agents may provide therapeutic benefit in PD and related synucleinopathies.

Poster Session 2 Abstracts

Decoding the functional interaction between MYRF and SOX10 in Oligodendrocytes

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Myelin is essential for rapid saltatory conduction in the nervous system. In oligodendrocytes, the myelination process is induced during terminal differentiation and is regulated by a complex network, which involves several transcription factors including Sox10 and the myelin regulatory factor (Myrf).

Myrf is a transcription factor that plays an important role during oligodendrocyte differentiation and maturation. Previous studies have investigated the molecular mechanism by which this transcription factor regulates the expression of myelin genes and reported that Myrf protein is activated by undergoing a posttranslational cleavage that separates the transcriptionally active N-terminal domain from a C-terminal region that retains Myrf in the membrane of the endoplasmic reticulum (ER). The transcription factor contains different domains including the N-terminal DNA binding domain (DBD), the central localized intramolecular chaperone domain (ICD) which mediates autoproteolysis, and a C-terminal transmembrane domain. After autoproteolysis, the N terminal fragment translocates as a homotrimer into the nucleus where the DBD can bind to regulatory elements of several oligodendrocyte-specific genes promoting maturation and myelination.

Previous observations revealed that Myrf expression does not occur in oligodendrocytes progenitors (OPC), but it is induced in the promyelinating stage of oligodendrocytes and is a direct target of Sox10.

Sox10 is another critical transcription factor that belongs to the SoxE subclass of high-mobility-group domain containing transcription factors together with Sox8 and Sox9. The SoxE factors are differentially expressed during oligodendrocyte development and are involved in the regulation of several processes, including oligodendroglial differentiation and CNS myelination.

When expressed Myrf cooperates with Sox10 in promoting oligodendrocyte myelination, as shown by the synergistic activation of myelin specific genes.

Here we study, whether Myrf additionally has an impact on genes that are regulated by Sox10 in OPCs, and whether it also interacts with the other SoxE factors Sox8 and Sox9. We also try to further dissect the functionally relevant structural features of Myrf.

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Adult neural stem cell/progenitor fate potential in vivo is controlled by COUP-TFI within the adult hippocampus

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In the adult hippocampal dentate gyrus (DG), adult radial-like neural stem cells (rNSCs) are multipotent (i.e. they generate both neurons and astrocytes) while progenitors are fate-restricted to the neuronal lineage. Interestingly, factors positively influencing neurogenesis, such as running, also increase DG astrogliogenesis, whereas pathological conditions, such as inflammation, alter the ratio of neuron and astrocyte production in favor of the latter one. Despite the importance of a tight control of neurogenic versus astrogliogenic potential, the underlying transcriptional program is still largely unknown.

In the healthy adult DG we found that a large subset of rNSCs/progenitors co-expressed the transcription factor COUP-TFI, whereas neuroinflammation leaded to its downregulation. By inducible knockouts and lineage tracing experiments we demonstrated that COUP-TFI deletion in adult DG rNSCs and committed neurogenic progenitors reduced neurogenesis and increased astrocyte production. Remarkably, this shift also occured upon COUP-TFI deletion by retroviral targeting of mitotic progenitors, indicating that COUP-TFI is required to repress astrogliogenesis all along the neurogenic lineage. Finally, by gain-of-function experiments we showed that COUP-TFI forced expression prevented astrogliogenesis in both normal and inflammatory conditions, indicating that COUP-TFI is a key transcriptional regulator driving adult DG rNSCs/progenitors towards a neurogenic fate by repressing an astrogliogenic one.

The role of TCF4 in adult neurogenesis of hippocampal dentate gyrus

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Intellectual disability is a condition characterized by significant limitations in adaptive and intellectual functioning (IQ<70), exhibiting a global prevalence of approximately 1%. In industrialized countries, ID has mostly a genetic origin and is caused by de novo mutations. Preclinical studies indicate that cognitive performance in intellectual disability can be impaired and enhanced by deficits, as well as stimulation of adult hippocampal neurogenesis, respectively.

The intellectual disability associated transcription factor 4 (TCF4) has been shown to be expressed in newly generated neurons of the adult hippocampus throughout all developmental stages of those. However, its function in adult neurogenesis remains to be determined. Here, we investigate the role of TCF4 in the generation of neurons in adult hippocampal dentate gyrus using a Tcf4 haploinsufficient mouse model. Our results suggest that Tcf4 haploinsufficiency reduces proliferation and survival of adult born neural cells. The subsequent evaluation of an enriched environment as a rescue factor shows an enhancement of the impaired neurogenesis in mutant mice. In the ongoing investigation, we address the role of Tcf4 in neuronal differentiation and morphology, and therein the effect of enriched environment.

Existing and upcoming results will contribute to our understanding of the highly common condition of intellectual disability and to the development of therapeutic options.

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The influence of α-synuclein oligomers on axonal integrity and cell loss in human stem cell-derived neurons

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Degeneration of the nerve cells in the midbrain and the protein deposits, which mostly consist of aggregated α-synuclein (α-syn), are the central pathological features of Parkinson's disease (PD). Mechanisms of midbrain neuronal cell death and pathomechanisms of α-syn aggregates in neurons are still largely unknown.

Here, we investigated and compared the cell death and the α-syn aggregation in the midbrain and cortical neurons in human PD cellular model using induced pluripotent stem cells (iPSC). Since axonopathy is one of the most critical early events in PD pathology, we further analyzed effects of α-syn small toxic aggregates, oligomers, on the axonal transport-regulating proteins in human iPSC-derived neurons.

IPSC-derived midbrain neurons from a PD patient carrying an α-syn gene duplication (Dupl) revealed an increased apoptotic rate and higher α-syn oligomer levels compared to cortical PD neurons and to control neurons. This coincidence might explain a selective vulnerability of midbrain neurons in human PD pathology. Moreover, reduced amount of proteins permitting mitochondrial axonal anterograde transport, KLC1 and Miro1, in neurites was associated with impaired anterograde axonal transport of mitochondria in human neurons with increased levels of α-syn oligomers.

Together, these data provide mechanistic insights into α-syn oligomeric toxicity to human neurons and could provide therapeutic targets by interfering with early disease pathology.

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Characterization of IPSC derived microglia-like (iMGL) cells to model Parkinson´s disease

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Neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, mostly are caused by neuronal death. Previously, it was hypothesized, that glial involvement is merely dependent on neuronal damage. Therefore, basic research focused on neuronal events to investigate and clarify underlying pathogenic mechanisms of these neurodegenerative disorders. As a main glial cell in the innate immune system of the central nervous system (CNS), microglial cells play critical roles in brain development, homeostasis, neurodegeneration, and brain aging. Microglial cells are resident macrophages of the central nervous system. Under physiological conditions, they are found in a resting state and capable to continuously scan their microenvironments to clear cell debris. These cells provide neurotrophic support to keep the surrounding neurons safe. In an activated stage of microglial cells, they can phagocytose invading pathogens and remove cell debris. By differentiating human induced pluripotent stem cells, we generated hematopoietic precursor cells (HPC). We stained these cells for CD34+, hematopoietic lineage marker, by flow cytometer. After that, we differentiated HPCs into primitive microglia like cells and then mature induced microglial like cells. We performed immunocytochemistry with specific microglial markers after primitive microglial differentiation. We also checked for transcriptional immune response markers of these cells with or without the treatment of LPS using real-time PCR. In next steps, we will investigate microglia like phenotypes and compare with different cell lines coming from the same origin (e.g. monocytes, macrophages) to characterize differentiated iMGL cells.

Fate mapping of adult hippocampal neural stem/progenitor cells in a model of neuroinflammation

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In the adult mouse hippocampus, newborn neurons and astrocytes arise throughout life from multipotent neural stem cells (NSCs) located in the subgranular zone of the dentate gyrus (DG). Neuroinflammation severely affects adult neurogenesis and increases astrocytes in the DG. However, few in vivo data are available concerning the effects of neuroinflammation on adult DG NSC/progenitor cell fate. In this study, we used two experimental approaches for fate mapping of DG NSCs and/or neuronal progenitors in an acute lipopolysaccharide (LPS)-induced neuroinflammation model.

First, we exploited the tamoxifen (TAM)-inducible Cre-LoxP system by using the Glast-CreERT2;Rosa26-floxed-stop-YFP mouse line. Interestingly enough, we found TAM treatment attenuated the neuroinflammatory response upon LPS treatment, indicating that this system, widely used in the field of adult neurogenesis, seems not suitable to study the early effects of neuroinflammation in adult neurogenic niches. Next, as alternative strategy we stereotaxically injected a retrovirus expressing the Cre-recombinase (RV-Cre) in the DG of Rosa26-floxed-stop-YFP mice followed by LPS treatment. RV-Cre efficiently targeted mitotic progenitors in the DG. Notably, in LPS-treated mice we observed a significant increase in newborn YFP+ astrocytes and reduced YFP+ neurons, compared to Saline-injected mice, providing direct in vivo evidence for neuron-to-astroglia shift in DG progenitor fate upon inflammation.

Neurogenic role of cystatin B in the etiopathogenesis of EPM1

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EPM1 is an autosomal recessive neurodegenerative disorder that has the highest incidence among the progressive myoclonus epilepsies worldwide. Loss of function mutations in the gene encoding CYSTATIN B (CSTB) are the primary genetic cause of EPM1. It is generally accepted that the loss of the antiprotease function of Cystatin B is the cause of EPM1 but the evidence supporting the involvement of CathepsinB is debatable.

Here we model EPM1 and the role of CSTB by using patient-derived cerebral organoids, a 3D model of human brain development. We are currently studying the expression of CSTB during the development of cerebral organoids and the potential role of its partner cathepsin B.

Interestingly, overexpression of CSTB and one of the pathological mutants show opposite effects on proliferation of human neural progenitors, suggesting a role of CSTB in regulating early steps of human neurogenesis and development.

We therefore collected blood samples from two patients with EPM1: one patient (UL1) homozygous for the most common EPM1 mutation, a dodecamer amplification in the promoter of CSTB gene; and one young patient (UL4), heterozygous compound with promoter amplification and point mutation in intron 1 causing exon 2 skipping. Starting from these 2 samples, we generated induced pluripotent stem cells (hIPSCs). Patients and controls IPSCs were then used to generate cerebral organoids and different cell population are currently under investigation using immunohistochemistry and FACS analysis.

The Influence of Ep400 on Schwann Cell Development in mice

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Schwann cells are the myelinating glia of the PNS. Development and differentiation of peripheral glia depends on transcription factors such as Sox10, Oct6 and Krox20 as well as chromatin remodelers such as Brg1 and Chd4. Many chromatin modifying factors have not yet been analyzed during Schwann cell development and myelination. One of those is the ATPase Ep400 which is a subunit of the Tip60/Ep400 chromatin remodeling complex. This complex fulfills its function mainly by exchanging histone H2A with its variant H2A.Z in promoter regions. Ep400 is known to be involved in differentiation processes, senescence and DNA damage response in various cell types. To analyze important functions of Ep400 in myelinating peripheral glia, we selectively deleted Ep400 in immature Schwann cells by Cre-mediated recombination. Postnatally, Ep400-deficient animals develop signs of hypomyelination and peripheral neuropathy with a reduction of mature, myelinating Schwann cells and myelinated axons. Loss of Ep400 provokes an increase in cell proliferation, cell death and immune response. GO analysis of differentially expressed genes in early postnatal sciatic nerves of control and Ep400-deficient mice confirms reduced expression of myelin genes. Current studies focus on the molecular mechanisms of Ep400 in Schwann cell development and myelination.

SoxC factors are required for cell survival during a specific time window of adult neurogenesis

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Adult neurogenesis is observed in two regions, the subventricular zone of the lateral ventricles and the hippocampal dentate gyrus (DG), of the mature murine brain. The generation of new neurons follows a stereotypic sequence of developmental cell stages, from radial glia-like neural stem cells, to neuroblast (NB)/ immature neurons (IMNs), to functionally integrated mature neurons. The transcriptional programs underlying these distinct developmental steps are orchestrated by transcription factor networks. In the DG, the expression of the SoxC group proteins Sox4 and Sox11 is required for NB generation and is maintained in IMNs. Moreover, the tightlycontrolled downregulation of Sox4 and Sox11 allows for IMNs to transition to functionally integrated dentate granule cell neurons (GCNs). Prior studies suggest that cells with prolonged Sox11 expression retain an immature electrophysiological and morphological phenotype. This precise temporal regulation prompts the question of which processes are regulated by SoxC during the stage of IMNs. We conditionally deleted Sox4 and Sox11 in NBs/IMNs in order to analyze their cellular function during their physiological expression period. Upon SoxC ablation, NB/IMN marker expression was drastically reduced. Fate analysis revealed that the reduction of NB/IMN marker expression was not the consequence of premature differentiation into mature GCNs, but due to loss of recombined cells. Our results indicate that SoxC factors are cell autonomously required for cell survival during a specific window of time in neuronal development. Further, upon inhibition of apoptosis, SoxCdeficient neurons continued to express pro-neuronal transcription factors and transitioned to a Calbindin-expressing cell stage. Neuronal morphology of the Calbindinexpressing cells, however, appeared rudimentary, which suggests an impairment of cytoskeleton dynamics in these cells We have also investigated the role of SoxC proteins in the second neurogenic niche, the SVZ. Stem cells in the SVZ mature info NBs/IMNs, which migrate along the rostral migratory stream towards the olfactory bulb. The mature neurons then integrate into the local neuronal network of the olfactory bulb. In contrast to the glutamatergic DG cells, these newly-born neurons display a GABAergic or dopaminergic neurotransmitter phenotype. Using the same conditional Sox4 and Sox11 deletion model, we observed impairment in neuronal maturation, resembling the phenotype observed in the hippocampal DG. In summary, we identified a SoxC-dependent critical phase during adult neurogenesis. The pro-survival function of SoxC factors is independent of the generated neuronal subtype and our results raise the possibility that SoxC factors control survival by establishing proper neuronal morphology and connectivity.

Alternative splicing regulates neurogenic commitment in the mammalian brain

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During embryonic development cells with an identical genetic information acquire different identities due to regulation of gene expression. Many research have focused on the genes which are activated or repressed during cell fate determination. However this approach does not take into account transcripts diversity originated from alternative splicing, therefore ignoring that a gene can give rise to multiple transcripts with different coding potential, hence function. Our aim to understand how alternative splicing regulates the switch from proliferative to differentiative division of neural stem cells, i.e the neurogenic commitment, in the mammalian developing brain in physiological condition. For this purpose we took advantage of a double transgenic mouse developed by our group that allows the isolation of proliferating progenitors (PP), differentiating progenitors (DP) and neurons, coexisting in space and time in the embryonic mouse cortex. Bioinformatic analysis on the transcriptome obtained from these cell populations allowed me to identify genes differentially spliced in the PP to DP transition. Most of the splicing events detected have an impact on the protein output of these genes. These data suggest that alternative splicing plays a critical role in neurogenic commitment in mammalian brain.

Role of C-terminal binding protein (CtBP1) in adult neurogenesis

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CtBP1 shuttles in an activity-dependent manner between the presynapse and the nucleus in neurons. In the nucleus it acts as a transcriptional co-repressor and chromatin modifier. With increasing neuronal activity CtBP1 exits the nucleus and shuttles towards the presynapse resulting in the removal of CtBP1-mediated transcriptional repression of its target genes. Existing studies provide evidence that CtBP1 plays a critical role in neurodevelopment, neurogenesis and neurodifferentiation. Importantly, a de novo missense mutation in the CtBP1 gene results in neurodevelopmental delay and intellectual disability in a cohort of unrelated patients. However, the mechanism by which CtBP1 affects neurodevelopment remains poorly defined.

In this project we characterize the role of CtBP1 in hippocampal adult neurogenesis. First, we assessed the stages of adult neurogenesis in which CtBP1 is expressed in brain sections from 9 weeks old mice by immunohistochemical labeling using a panel of neurogenesis markers including Nestin, Mcm2, Tbr2, DCX, and Prox1. We detected CtBP1 expression in sparse Nestin-positive radial glia-like stem cells (type 1) but not in Nestin-positive early progenitor cells indicating a down-regulation of CtBP1 in the early differentiation steps of neuronal precursors. Furthermore, we demonstrate a co-localization of CtBP1 with either Mcm2 or Tbr2 in intermediate progenitor cells (type 2b and 3) but not in all Mcm2 or Tbr2 positive cells (type 2a). Interestingly, CtBP1 co-localized with Mcm2 and Tbr2-positive nuclear clusters, but not to heterochromatin preferentially stained by DAPI, suggesting a co-recruitment of these transcriptional regulators to identical euchromatin domains. Labeling of CtBP1 in DCX and Prox1-positive cells indicates an expression of CtBP1 also in the postmitotic immature neurons. Finally, we tested the effect of CtBP1 knockout on hippocampal adult neurogenesis. These data will shed new light on the mechanism by which CtBP1 influences neurodevelopment and adult neurogenesis.

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C1-esterase inhibitor treatment prevents blood-brain barrier dysfunction in the neonatal mouse brain after acute systemic hypoxia

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Background: Hypoxic-ischemic complications are the major cause of brain lesions during the perinatal period. The vulnerable BBB and the post-hypoxic brain oedema are putative therapeutical targets. Here, we evaluate the ability of C1-INH to provide neuroprotection in the hypoxic neonatal mouse brain.

Methods: P7-C57BL6/NCrl mice were exposed to normoxia or hypoxia (8% O_2). After re-oxygenation, mice were injected (i.p.) with 7.5–30 IU/kg C1-INH (CSL Behring). Tissue preparation was performed after 24h. Cerebral mRNA expression of pro-apototic genes, matrix metalloproteinases (MMP), MMP inhibitors (MMPI), and tight junction proteins was analyzed, CC3- and TUNEL-staining was performed, and protein levels of S100b and albumin was quantified.

Results: In hypoxic control mice bnip3 and dusp1 expression as well as the number of apoptotic cells was increased, whereby cerebral and plasma S100b was elevated two- and 32-fold, respectively. C1-INH treatment abolished hypoxia-increased expression of pro-apoptotic genes and elevated numbers of CC3- and TUNEL-positive cells in the brain of hypoxia-exposed mice. C1-INH prevented elevated S100b protein levels and abolished passage of albumin from blood to the brain parenchyma in hypoxia-exposed mice. Furthermore, a dose-dependent increase of ZO-1 and Occludin mRNA was observed, while changes in mRNA expression levels of other tight junction proteins, MMP, and MMPI were not detected.

Conclusions: We demonstrated that C1-INH treatment stimulates mRNA expression of different tight junction proteins, abolishes escalating plasma levels of S100B, and impairs hypoxia-induced apoptotic signal cascades in hypoxic mice. Suggesting a BBB stabilizing, neuroprotective effect, supplemental C1-INH therapy shows promise as a supportive neuroprotective treatment option.

Zooming in on cryopreservation of hiPSCs and neural derivatives: a dual-center study

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Human induced pluripotent stem cells (hiPSCs) are an important research tool and efficient cryopreservation is a major challenge. The current gold standard for hiPSCs is slow-rate freezing in suspension, but low recovery rates are limiting immediate post-thawing applicability. We tested whether the switch from slow-rate freezing to ultra-fast cooling by vitrification improves post-thawing survival in a selection of hiPSCs and small molecular neural progenitor cells (smNPCs) from Parkinson's disease (PD) and controls. In a dual-center study, we compared the results by immunocytochemistry (ICC) and fluorescence-activated cell sorting (FACS) analysis. Moreover, RNA-sequencing (RNA-seq) before and after freezing was performed. Adherent vitrification was achieved in the so-called TWIST substrate, a device combining cultivation, vitrification, storage, and post-thawing cultivation. Vitrification resulted in preserved confluency and significantly higher post-thawing cell numbers and viability at day one after thawing, while no significant results were obtained at day four after thawing. RNA-seq and ICC of hiPSCs revealed no change in gene expression and pluripotency markers after cryopreservation, indicating that physical damage after slow-rate freezing disrupts the cellular membranes. Scanning electron microscopy (SEM) revealed preserved colony integrity and intact cell-cell adhesions by adherent vitrification. Experiments in smNPCs demonstrated that adherent vitrification is also applicable to neural derivatives of hiPSCs. Our data suggest that, compared to the state-of-the-art slow-rate freezing in suspension, adherent vitrification is an improved cryopreservation technique for hiPSCs and derivatives.

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Probing the transcriptional programs underlying human neurogenesis by direct lineage reprogramming

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Ectopic expression of defined transcription factors can force direct cell fate conversion from one cell lineage to another in the absence of cell division1. Several transcription factor cocktails have enabled successful reprogramming of various somatic cell types into induced neurons (iNs) of distinct neurotransmitter phenotype2-5. Analyses of the transcriptome alterations induced by the reprogramming factors has yielded fundamental insights into the molecular mechanisms of iN conversion6-9. However, the intermediate states that drive the reprogramming trajectory towards distinct types of iNs are largely unknown. Reconstructing such trajectory using single-cell RNA-seq, here we show that successful reprogramming of adult human brain pericytes into functional iNs by Ascl1 and Sox2 (AS) encompasses activation of a neural stem cell-like gene expression program that precedes bifurcation into distinct neurotransmitter lineages. Intriguingly, the neural stem cell-like program included transient expression of several signaling pathway modulators such as the BMP inhibitor Noggin. While many of the AS-iNs failed to mature, enhancing BMP inhibition during early phases of reprogramming promoted the advancement through the bifurcation fork that splits the iN trajectory into DLX- or NEUROG2-dominated paths, suggestive of diversification into inhibitory and excitatory neuron subtypes. Our results show that AS-mediated reprogramming into a broad spectrum of iN types involves the unfolding of a developmental program that encompasses neural stem cell-like intermediates. The identification of the molecular programs that establish cellular intermediates during iN reprogramming provides handles to improve lineage conversion towards therapeutically relevant cell types.

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Expansion of the radial glia-like stem pool in the hippocampal dentate gyrus is modulated by the chloride importer NKCC1

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The neurogenic capacity of the hippocampus is dependent on the existence of a population of radial glia-like cells (RGLs) that act as neural stem cells. However, the underlying mechanisms regulating RGL activation throughout life have not been fully elucidated. The inhibitory neurotransmitter GABA, acting via $GABA_A$ receptors, regulates multiple stages of adult neurogenesis. The mode of GABA action depends on intracellular chloride levels, which are determined by the differential expression of chloride importers NKCC1 and KCC2. NKCC1 is predominantly expressed in neural precursor cells and drives Cl^- influx. The role of the chloride importer NKCC1 in the activity of RGLs from the dentate gyrus remains unknown. Therefore, we used a novel inducible transgenic mouse model for specific NKCC1 knock-out in $nestin^+$RGLs. Our study shows that the self-renewal capacity of RGLs in the adult and aged hippocampal dentate gyrus is strongly modulated by the chloride importer NKCC1. Understanding the underlying mechanisms of neural stem cell activation throughout life is important to restitute the stem cell pool and generate new neurons, thereby improving the cognitive function during aging.

Disease-specific alternative splicing events in ALS are associated with TDP-43 binding

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder affecting cortical and spinal motor neurons (MNs) leading to severe muscle wasting and ultimately death due to respiratory failure. Post mortem, the disease is characterized by the cytoplasmic mislocalized and biochemically altered RNA-binding protein TDP-43 and a dysregulated alternative splicing (AS) network. TDP-43 has been implicated in AS, but a causal relationship between disease-related AS events and pathological alternations in TDP-43 has not yet been established. Here, we used induced pluripotent stem cells (iPSC) from ALS patients, differentiated them into MNs and performed subsequent analyses: Although we were not able to observe mislocalization of TDP-43, the protein showed increased insolubility and phosphorylation in ALS MNs. Furthermore, more than 1000 AS events can be detected by RNA-sequencing. To investigate if the AS events are linked to TDP-43 binding in the flanking introns on a global level, we performed enhanced cross-linking and immunoprecipitation sequencing (eCLIP-seq) of TDP-43 in iPSC-derived MNs. Interestingly included exons, but not exons that are excluded in ALS have a higher TDP-43 binding density in the upstream as well as the downstream intron. In summary, using our iPSC-based model, we are able to detect a large number of pathological AS events that are associated with TDP-43 even before the full extent of TDP-43 pathology seen in post mortem tissue is reached. Therefore, TDP-43-realted, pathological AS is most likely an early, potentially pre-symptomatic characteristic of the disease. Follow-up studies will reveal the detailed mechanism behind this observation and identify target events with diagnostic value and the potential of therapeutic intervention.

Stress impedes neuronal differentiation via ZBTB16 in human brain organoids

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Psychological stress is an important contributing factor in the onset and progression of many psychiatric disorders such as depression and anxiety. Stress results in the activation of the stress-hormone-regulating hypothalamic-pituitary-adrenal (HPA) axis, which has been implicated extensively in the causality and the treatment of depression. ZBTB16 (zinc finger and BTB domain containing 16) is a stress – response transcription factor that regulates the expression of many glucocorticoid – response genes and has been reported to have a neuroprotective role. Single nucleotide polymorphisms in ZBTB16 have been extensively linked to mood and anxiety disorders, autism and schizophrenia. We hypothesize the glucocorticoids impede the neuronal differentiation pathway and that this trajectory change is mediated via ZBTB16.At the RNA level, ZBTB16 is dynamically expressed in human brain organoids picking at the early stages around day 30. ZBTB16 is co-expressed with neuronal progenitor cells' markers and is reduced when the neuronal markers arise. At the protein level in human brain organoids from 2 different iPSCs lines ZBTB16 is expressed in the basal side of the SVZ. Stress through dexamethasone treatment increases ZBTB16 expression in the basal radial glia and results in decreased neuronal differentiation as shown both in 2D and in 3D human neuronal cultures. In conclusion, stress during neurodevelopment alters the neuronal differentiation pathway via ZBTB16. This could pose a potential mechanism on how stress during development affects the progression of mental diseases in adulthood.

alpha-Synuclein deficiency in neuroinflammation and demyelination in models of multiple sclerosis

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In neurodegenerative and –inflammatory diseases, such as multiple system atrophy and multiple sclerosis (MS), loss of myelin and activation of immune responses are associated with aggregation of alpha-Synuclein (aSyn). However, its exact contribution to inflammatory demyelination is still elusive. Here, we aim to study the effect of aSyn deficiency in neuroinflammation as well as de- and regenerative processes in the context of MS by using two different animal models. First, we investigated the role of aSyn in experimental autoimmune encephalomyelitis (EAE), which resembles inflammation and demyelination in the central nervous system (CNS) as observed in MS patients. Wildtype ($aSyn^{+/+}$) and aSyn-deficient ($aSyn^{-/-}$) mice were monitored for 8 weeks. $aSyn^{-/-}$ mice showed an ameliorated disease course compared to littermate controls with wildtype aSyn expression (*$p<0.05$, n=12/11 per group). At the peak of motor dysfunction, these mice exhibited mild gait ataxia, while the control group suffered from moderate paralysis of hind limbs. Histological analysis of CNS infiltrating immune cells revealed a significantly lower number of $CD3^+$ cells in spinal cord lesions of $aSyn^{-/-}$ mice compared to $aSyn^{+/+}$ mice (101 vs.236 $CD3^+$ cells, ***$p<0.001$, n=5 per group). To assess the impact of aSyn on oligodendrocyte pathology in the absence of neuroinflammation, we employed the cuprizone (CPZ) model. After 5 weeks of CPZ diet, extent of demyelination in the corpus callosum was assessed by immunohistochemical staining for myelin proteins. Here, no differences in myelination scores were observed between $aSyn^{-/-}$ and $aSyn^{+/+}$ mice (n=7 per group). Summarizing, our data suggest the involvement of aSyn in neuroinflammatory rather than demyelinating processes in the CNS.

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The extracellular matrix protein LGALS3BP regulates basal radial glial cells generation and human cortical development

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The human brain is characterized by increased number of neurons generated during fetal neurogenesis resulting in the expansion of its total size and emergence of gyri. This increase is a direct consequence of the basal radial glial progenitors (bRGCs), which reside in the outer subventricular zone and are mainly depicted in the cortex of primates and humans. The molecular and cellular mechanisms regulating their generation and function are largely unknown. Towards identifying new molecules interplaying in bRGCs generation and function, we sought to investigate the role of LGALS3BP, an extracellular matrix protein which was found to be enriched in human bRGCs.

We show that manipulation of LGALS3BP expression in cerebral organoids, in human fetal brain or in the developing mouse cortex results in disruption of the apical junction, increased cell delamination and changes the neuronal outcome, which regulated eventually the gyrification of the human cortex.

Our data show that the molecular mechanisms involve several ECM and tetraspanin proteins and suggest LGALS3BP as a key regulator of the differentiation of aRGCs to bRGCs, bRGCs delamination and human corticogenesis.

Impact of the ASM/ceramide system on hippocampal neuronal excitability

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The prevalence of major depressive disorder (MDD) is more than 10% over the world population. The acid sphingomyelinase (ASM)/ceramide system has been advanced as a major player in the pathogenesis of MDD. Indeed, the activity of ASM is enhanced in MDD patients, with higher concentrations of plasma ceramide than in healthy individuals. Here, we used wild type (WT) and ASM knockout (koASM) mice to explore the impact of the ASM/ceramide system on hippocampal neuronal excitability. Whole-cell patch-clamp recordings were performed on hippocampal cells (CA1 pyramidal cells and dentate gyrus granule cells) in brain slices from adult WT and koASM mice. Our preliminary data show no significant differences in neuronal excitability between WT and koASM hippocampal cells. However, the specific ASM inhibitor ARC39 (1 µM) strongly reduced the excitability of neurons from ventral hippocampus of WT mice. No such inhibitory effect of ARC39 was observed in koASM pyramidal cells, demonstrating the drug´s specific action on ASM. Application of C2-ceramide produced mixed effects on cell excitability along the hippocampal longitudinal axis. The C2-ceramide-induced excitation in dorsal CA1 pyramidal cells (71%) was shifted towards inhibition in ventral CA1 pyramidal cells (only 22% excitation). In dorsal granule cells, the uniformly excitatory effect of ceramide (100%) in WT mice was altered in koASM mice (69%), suggesting that the reduced ceramide level in koASM mice might alter the responsiveness to ceramide. Furthermore, voltage-clamp recordings from granule cells revealed that the ceramide-induced apparent inward currents reversed around -90 mV, close to the reversal potential of potassium channels. Our data suggest that the ASM/ceramide system exerts a complex pattern of acute electrophysiological effects in the hippocampus, depending on which subregion along its longitudinal axis is under study.

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P19: A novel interactor in neural progenitor cells and microglia crosstalk?

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Despite decades of research in the genetic regulation of brain formation, the role of several genes remains unknown. Our lab has identified an uncharacterized gene, P19, that during brain development is physiologically upregulated in differentiating progenitors compared to both neural stem cells and neurons. To investigate the role of this novel factor in cell fate commitment, we overexpressed P19 by in utero electroporation finding a reduced proportion of intermediate (Tbr2+/Btg2+) progenitors in the SVZ, and less new-born neurons reaching the cortical plate. In addition, we detected an increase in cell cycle exit; concomitantly with ectopic new-born neurons in the VZ/SVZ. Interestingly, these phenotypes were detected not only within transfected cells, but also in untransfected ones suggesting cell-extrinsic effects by P19. In line with this, microglia cells (Iba1+) were found to cluster within the P19 transfected area. While the role of microglia as immune cells of the adult brain is well known, their contribution to embryonic neurogenesis remains elusive. Further experiments will investigate whether P19-recruited microglia cells are responsible for the phenotypes observed and their crosstalk with neural progenitors.

Autophagy inhibition promotes alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophago-exosome phenotype

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The autophagy-lysosome pathway (ALP) regulates intracellular homeostasis of the cytosolic protein alpha-synuclein, and is impaired in synucleinopathies, including Parkinson´s disease and dementia with Lewy bodies (DLB). Emerging evidence strongly suggests that the ALP also influences alpha-synuclein release, but this remains mechanistically unresolved. Several studies identified alpha-synuclein in exosome/extracellular vesicle (EV) fractions. EVs generated in the multivesicular body compartment are either released upon its fusion with the plasma membrane, or cleared via the ALP. We therefore hypothesized that inhibiting ALP clearance 1) enhances the extracellular shuttling of alpha-synuclein and additional multivesicular body contents via EVs, 2) alters EV biochemical profile, and 3) promotes alpha-synuclein cell-to-cell transfer. We demonstrate that ALP inhibition increases the ratio of extra- to intracellular alpha-synuclein and upregulates alpha-synuclein association with EVs in neuronal cells. Ultrastructural analysis revealed a widespread, fused multivesicular body-autophagosome compartment. Biochemical characterization indicated the presence of putative autophagosome markers, including LC3-II and p62, within EVs. This distinct “autophagosome-exosome” profile was also identified in human cerebrospinal fluid (CSF) EVs. After a single intracortical injection of alpha-synuclein-containing EVs derived from CSF into mice, human alpha-synuclein colocalized with neuronal endosomes. Prominent alpha-synuclein immunoreactivity and a higher number of neuronal alpha-synuclein inclusions were observed after DLB patient CSF EV injections. In summary, this study provides compelling evidence that a) ALP inhibition increases alpha-synuclein within neuronal EVs, b) EVs contain putative ALP components, and c) CSF EVs transfer alpha-synuclein from cell to cell in vivo. Thus, autophagy may regulate EV protein composition and consequently progression in synucleinopathies.

Beyond the Flow: The Role of Epithelial Sodium Channel in Neural Cells

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The electrochemical gradient between the extracellular and intracellular space represents an important signaling element in stem cell biology. We have shown that the voltage and ligand independent, constitutively active sodium epithelial channel (ENaC) regulates proliferation of the adult neural stem cells (aNSCs) and their progeny (Petrik et al., 2018). Our results suggest that the mechanosensitive ENaC allows the aNSCs at the surface of the lateral ventricle to sense a fluid flow as a cue that can trigger their proliferation. However, ENaC is regulated by many mechanisms other than the fluid shear stress and is expressed also outside the adult neurogenic niche. Here, we will present a work-in-progress of investigating the possible role of ENaC during embryonic development, in astrocytes, and in the retina.

Reference: Petrik et al.: “Epithelial Sodium Channel Regulates Adult Neural Stem Cell Proliferation in a Flow-Dependent Manner.” Cell Stem Cell, 22(6), 865-878, 2018.

Exploring the cellular and molecular mechanisms of the neurodegenerative phenotype in hereditary spastic paraplegia type 11

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Hereditary spastic paraplegia (HSP) is a heterogeneous group of rare motor neuron disorders characterized by progressive weakness (paraplegia) and spasticity of the lower limbs. HSP type 11 (SPG11), the most common form of complex autosomal recessive HSP, is accompanied by a thin corpus callosum (TCC), intellectual disability, dysarthria, sensory and motor neuropathy, and amyotrophy. The wide range of symptoms can be further categorized into neurodevelopmental and neurodegenerative phenotypes. Whereas our previous studies have extensively addressed the neurodevelopmental impairments of the disorder, the neurodegenerative phenotype of the disease remains largely unknown. In order to address this aspect, we employed spg11 and control human iPSCs-derived cortical neurons. First, we discovered that neuritic pathology and increased cell death of patient-derived neurons could be reverted by addition of the GSK3 inhibitor, tideglusib. In order to delve deeper into the mechanistic pathology of the disorder and based on transcriptome analysis that pointed towards alterations in lipid metabolism, we performed lipidomics analysis and found that spg11-patient derived neurons express higher level of hexosyl ceramides. Our aim is to perform further molecular and biochemical assays for elucidation of the observed lipid alterations. In addition, due to a lack of reliable antibodies for spg11, we are currently using CRISPR/Cas9 technique for generation of spg11 hES reporter line which will be employed for validation of co-localization with candidate proteins. Determination of the specific lipids accumulated in the disease as well as the elucidation of the involved pathways is critical for a precisely targeted therapeutic approach.

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The role of alpha-synuclein oligomers and neuroinflammation in human pathology of Parkinson's disease

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Parkinson´s disease (PD) is neurodegenerative disorder characterized by the progressive degeneration of midbrain neurons (MN). An accumulation of protein aggregates mainly consisting of alpha-synuclein (aSyn) and inflammation are suggested to play a crucial role for neurodegeneration in PD. However, the mechanisms of their contribution to neuronal loss and their possible interplay during PD pathology remain elusive.

Small aSyn aggregates, oligomers, are characteristic for early PD pathology and are considered the most toxic species to neurons. aSyn oligomers were identified to disrupt interplay of main axonal transport components, microtubules and kinesin, in a cell-free assay. Furthermore, significant disruption of axonal transport was measured in induced pluripotent stem cell (iPSC)-derived human MN characterized by high levels of aSyn oligomers, including iPSC-derived MN from a PD patient with aSyn gene duplication. Axonal transport defects could be rescued by using a de novo synthesized compound known to inhibit aSyn oligomer formation.

A potential role of adaptive immune system for neuroinflammation in PD has been suggested. We show that T cells induce MN death in sporadic PD by IL-17, upregulation of IL-17 receptor and NFkB activation using human autologous co-culture of peripheral T cells and iPSC-derived MN. Higher Th17 frequencies were also evident in the blood of PD patients and increased numbers of T cells were detected in postmortem PD brain tissues. Blockage of IL-17 or IL-17R rescued the neuronal death.

Since IL-17 can influence axonal transport, aSyn-induced axonopathy might precede Th17-induced neuronal death in human PD pathology. This connection needs to be further investigated.

Antidepressant drugs require astrocytes to prime an early synaptic pruning and remodelling in the prefrontal cortex

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Astrocytes are non-professional phagocytes that engulf synapses to remodel neuronal circuits during postnatal developmental stages. Neuropsychiatric disorders such as major depressive disorder (MDD) are characterized by deficits in synaptic communication and neuronal connectivity, which can be reversed by antidepressants (ADs). This suggests a role for astrocyte-mediated phagocytosis in the pathogenesis of these disorders and/or in response to ADs. Aim of this work was to identify astrocyte-dependent molecular triggers of MDD and examine their impact on neuronal synapses upon AD treatments. Recently, MEGF10 emerged as a mediator of astrocyte-dependent synapse elimination in the developing mammalian brain. MEGF10 activation depends on the induction of the MAPK/ERK1/2 pathway, a regulator of cellular plasticity. Our results showed that

treatment of astrocytes and neurons with different antidepressants (ADs) led to opposite ERK1/2 activation patterns and a “re-juvenalization” effect in these two cell types. Furthermore, we observed a reduction in synaptic densities in neurons after 48h AD treatment, but only in the presence of astrocytes. This astrocyte-dependent synaptic pruning also occurred in the adult rat prefrontal cortex after 48h treatment with the AD fluoxetine and correlated with increased MEGF10 expression. However, no differences in MEGF10 were evident in the adult brain of an animal model of depression. We therefore propose that ADs favour the remodelling of neuronal circuits in the adult brain by reactivating a ‘’juvenile-like plasticity program’’ characterized by an MEGF10-dependent astrocyte-mediated synaptic reshaping. Finally, we suggest MEGF10 as a pharmacological target for the development of drugs aimed at rescuing synaptic aberrancies.

FoxO-dependent autophagic flux controls development of the postsynaptic compartment of adult-generated hippocampal neurons

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Autophagy is a highly conserved catabolic pathway with emerging functions in adult neurogenesis. The mechanisms controlling autophagy in adult generated neurons are largely unknown. Here we identify transcription factors of the FoxO family as central regulators of autophagy in adult hippocampal neurogenesis. We found that conditional deletion of FoxO transcription factors strongly impaired autophagic flux in developing neurons of the adult hippocampus. Furthermore, FoxO-deficiency altered dendritic morphology, elevated spine density and led to aberrant spine positioning in adult-generated hippocampal neurons. Strikingly, pharm FoxO-dependent autophagic flux controls development of the postsynaptic compartment of adult-generated hippocampal neurons.

Dendritic and synaptic structural plasticity in adult newborn granule cells

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Aims: Here we study structural maturation, synaptic integration and plasticity of adult newborn GCs (abGCs) in young adult (8-13 week of age) rats in vivo.

Methods: We injected a retrovirus to label abGCs and an adeno-associated virus to label mature GCs (mGCs). Structural analysis were combined with in vivo high-frequency stimulation (HFS) of the medial perforant path known to induce homosynaptic LTP (hom-LTP) and simultaneous heterosynaptic LTD (het-LTD). [1,2,3,4]

Results: Complete reconstructions from abGCs from 21-77 days post intrahippocampal injection (dpi) exhibited significant structural differences to mGCs. Following 2h HFS we found a homosynaptic spine head enlargement in the stimulated MML and heterosynaptic spine head shrinkage in the adjacent OML gradually in abGCs between 28 dpi and 35 dpi. Application of the non-competitive NMDA receptor antagonist MK-801 abolished hom-LTP and het-LTD and structural spine changes [4]. We further correlated GC morphologies with HFS responsiveness using the immediate-early gene Arc as a marker of synaptic activation.[1] Only abGCs at 28 and 35 dpi but neither old abGCs nor mGCs responded to stimulation with a remodeling of their dendritic arbor. [2]

Based on our structural analysis and on the GC ion channel composition known from intracellular studies we developed a novel compartmental model of GCs[3]

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The Parkinson`s disease relevant stressor Rotenone triggers senescence in human astrocytes

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One major risk factor for Parkinson`s disease is aging. Core pathomechanisms of PD, such as oxidative stress and mitochondrial dysfunction, are also increased with age and even healthy subjects exhibit aging related neurodegeneration in the substantia nigra. Senescent cells accumulate with age, they accelerate aging in organs and they are linked to aging related diseases such as Sarcopenia or Arteriosclerosis. In addition to aging, which strongly connects PD and senescence, oxidative stress and mitochondrial dysfunction are closely linked to both, PD pathology and senescence induction. The hypothesis of this study is that there is increased senescence in PD patients which accelerates the disease progression.
We therefore aim to analyze whether the PD relevant stressor Rotenone is able to induce a senescent phenotype in human astrocytes. For this, fetal astrocytes were exposed to Rotenone and senescence markers such as Interleukin-6 (IL-6), senescence-associated beta-galactosidase (SA-b-Gal) and the CDK-inhibitor p16 were analyzed. We were able to measure an increase in senescence markers within cells treated for short term as well as long term with Rotenone. An increase in IL-6 might indicate a proinflammatory effect of senescent astrocytes in the brain with higher inflammation being one pathomechanism of PD.
mTOR signaling is known to promote senescence and mTOR inhibition reverts senescence phenotypes and extends lifespan in model organisms. We were able to measure a decrease in SA-b-Gal with mTOR-inhibition showing that Rotenone-induced senescence depends on mTOR activity.
Glial fibrillary acidic protein (GFAP) is a marker for reactive astrocytes and we could measure a decrease in GFAP expression in senescent astrocytes hinting at a difference between senescent and reactive astrocytes. In further experiments we will analyze the transcriptome of senescent astrocytes and compare it to reactive A1 astrocytes which will be generated by treating the cells with tumor necrosis factor alpha (TNFa) and Interleukin-1 alpha (IL-1a). Moreover we will analyze how senescent astrocytes affect neural function. Astrocyte senescence might impair processes related to neuroprotection, neurotoxicity, transmitter release or blood-brain-barrier function and in this manner contribute to disease progression.

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Fragmentation of Huntingtin in the context of neuronal development and inflammation in a human cellular model of HD

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Huntington's disease (HD) is a fatal neurodegenerative disorder caused by extended CAG repeats within the coding region of the huntingtin gene. HD pathology is predominantly brain specific with prominent neurodegeneration of striatal medium spiny neurons (MSNs). In addition, hyperactivation of microglial cells was observed in HD patient brains and mouse models promoted by the accumulation of a toxic exon 1 fragment of mutated Huntingtin leading to neuronal expression of IL-34. Therefore, we hypothesize that N-terminal toxic mHTT fragments alter cellular biology of MSNs during neurodevelopment, and hyperactivity of human monocytes and microglia is an early response to mHTT toxicity. To this end, we have established a human cellular HD model using induced pluripotent stem cells (hiPSC). Fibroblasts from HD patients (n=4; CAG repeats 15/39, 19/42, 17/54 and 29/59) and healthy controls (n=4) were reprogrammed to hiPSCs using the Yamanaka factors and further differentiated into neural precursor cells. Ultimately, we were able to obtain approximately 40% differentiated neurons co-expressing the neuronal marker βIII-Tubulin and importantly, the putative MSN marker dopamine- and cAMP-regulated neuronal phosphoprotein 32. In the light of antisense technology it is important to understand the time point of mHTT toxicity and underlying pathways preventing and/or promoting neurodegenerative processes. The established human cellular disease model of HD will be the basis to examine the time point of mHTT fragmentation during neurodevelopment. Furthermore, potential early mHTT toxicity and (NF)κB pathway related hyperactivity of human monocytes and microglia will be assessed in an autologous human co-culture cell model of HD.

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Sox11 dosage controls early neuronal fate and neurite morphology in a human Coffin Siris syndrome like syndrome model

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Sox11 - a member of the SoxC transcription factor family - is a transcriptional regulator of neurodevelopment. Analyses of murine transgenic models indicate that SOXC transcription factors have highly redundant functions in mouse development, which may explain the observation that haploinsufficiency for a single SOXC-factor does not result in obvious phenotypes. It is therefore surprising that in humans, haploinsufficiency of SOX11 can cause Coffin-Siris syndrome like syndrome (CSS like syndrome), a rare neurodevelopmental disease characterized by intellectual disability, microcephaly and hypoplastic nails, warranting further investigation of the function of SOX11 in human neurodevelopment. To this end, we generated a $SOX11^{+/-}$ model using human embryonic stem cells (hESC) with CRISPR/Cas9 genome engineering. NPCs derived from clonal $SOX11^{+/-}$ hESCs had a 50% reduced SOX11 protein expression.

Differentiation of the SOX11 haploinsufficient NPCs into cortical neuronal cells revealed significantly lower fractions of neuroblasts and immature neurons early after induction of differentiation suggesting that SOX11 dosage controls efficacy of neuronal differentiation of human NPCs. We further found significantly shorter average neurites and less complex dendrites suggesting SOX11 dosage as a key regulator of neurite growth.

To further understand the effects of SOX11 haploinsufficiency during human neurodevelopment and its role in the pathogenesis of Coffin-Siris syndrome like syndrome we will study alterations in neuronal network formation by using multielectrode array technology. Overall, this study will help to understand molecular features of neurodevelopmental diseases such as Coffin-Siris syndrome like syndrome and broaden knowledge of SOX11 function in human disease.

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Id4 eliminates the pro-activation factor Ascl1 to maintain quiescence of adult hippocampal stem cells

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Quiescence is essential for the long-term maintenance of adult stem cells and tissue homeostasis. However, how stem cells maintain quiescence is still poorly understood. We have found that stem cells in the dentate gyrus of the adult hippocampus actively transcribe the pro-activation factor Ascl1 regardless of their activation state. In neural stem cell cultures, we found that the inhibitor of DNA binding protein Id4 is responsible for the elimination of Ascl1 protein. Id4 sequesters Ascl1 heterodimerisation partner E47, promoting Ascl1 protein degradation and neural stem cell quiescence. Accordingly, elimination of Id4 from stem cells in the adult hippocampus results in abnormal accumulation of Ascl1 protein and premature stem cell activation. We also found that multiple signalling pathways converge on the regulation of Id4 to reduce the activity of hippocampal stem cells. Id4 therefore maintains quiescence of adult neural stem cells, in sharp contrast with its role of promoting the proliferation of embryonic neural progenitors.

Neuronal EGFL7 affects hippocampal neurogenesis with consequences for learning and memory

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Hippocampal neural stem cells (HC-NSCs) residing in the subgranular zone (SGZ) of the dentate gyrus (DG) give rise to newborn neurons throughout life in mice and thus affect learning and memory. The regulatory mechanisms of adult hippocampal neurogenesis have not fully been unraveled, therefore we investigated the role of the epidermal growth factor-like protein 7 (EGFL7), a secreted neurovascular protein and a non-canonical Notch ligand[1,2]. In this work we report that EGFL7 is expressed in the DG of the hippocampus by endothelial and granule cells as detected by IF and qRT-PCR of diverse FACS-sorted DG cell types. Constitutive loss of EGFL7 in knock-out mice induced an activation of NSCs as shown by the upregulation of progenitor markers (e.g., Stathmin1 and Mash1). Mechanistically, loss of EGFL7 upregulated cytokines that are responsible for proliferation and cell cycle regulation. As a consequence of this activation, more adult born neurons formed in vivo. The analysis of DG dendritic spines in EGFL7 knock-out mice crossed with Thy1-GFP-M revealed that upon the loss of EGFL7, neurons exhibited an increased dendritic complexity. Upon loss of EGFL7 in neuronal cells of the CamKIIa-Cre mice, similar phenotype characteristics to those of constitutive EGLF7 knock-out mice were observed. Finally, mice deficient for EGFL7 performed better in the Morris water maze and IntelliCage showing enhanced spatial memory. In conclusion, our findings suggest EGFL7 as a negative feedback mechanism applied by mature granule cells, allowing them to tightly control adult neurogenesis. These findings have implications for learning and memory even upon high age.

The role of Tcf4 in oligodendrocyte development

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Oligodendrocytes are the myelinating glia in the central nervous system (CNS). The differentiation from oligodendrocyte precursor cells (OPC) to myelinating oligodendrocytes (mOL) depends among other factors on the expression of class II basic Helix-Loop-Helix (bHLH) proteins such as Olig2 and Mash1. Class II bHLH proteins are known to form heterodimers with class I bHLH proteins. Tcf4, a class I bHLH transcription factor, is expressed at substantial levels in the oligodendroglial lineage. To investigate the function of Tcf4 during oligodendrocyte development, we analyzed a constitutive Tcf4 knockout model at embryonic stages. At 18.5 dpc, Tcf4-deficient knockout mice show a drastic reduction in the expression of myelin related genes such as Myrf, Mbp and Plp1 whereas OPC markers appear normal. This indicates that Tcf4 is important for the terminal differentiation of oligodendrocytes and might therefore be an essential heterodimerization partner for one or several class II bHLH factors. Current studies focus on the underlying molecular network and the identification of potential class II bHLH interaction partners.

Modeling alpha-synucleinopathy in human IPSC-derived oligodendrocytes

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Accumulation of alpha-synuclein (α-syn) within oligodendroglial cytoplasmic inclusions (GCIs) is a main pathological hallmark of the age-related atypical parkinsonian disorder multiple system atrophy (MSA). While these α-syn inclusions are strongly associated with myelin loss, causal pathomechanisms leading to MSA pathology are still poorly understood. Recent data from rodent studies indicate α-syn as an inhibitor of oligondendroglial maturation and myelination, however, little is known about its impact on human oligodendrocytes. In order to understand the molecular mechanisms of α-syn aggregation in human oligodendrocytes, we aim to establish an in vitro model of MSA using human induced pluripotent stem cells (hIPSC). Upon Sendai reprogramming, neural progenitor cells (NPCs) were generated and further differentiated into human oligodendrocytes initially expressing O4 followed by myelin basic protein (MBP) expression. In vitro modelling of MSA will be achieved by a dual approach: (1) a constitutively cell-autonomous expression of α-syn and (2) the exposure to exogenous recombinant α-syn. In addition, we will establish a temporal-controlled endogenous expression of α-syn. Besides analyzing potential effects of intra- and extracellular α-syn on myelination, we are particularly interested in elucidating the impact of α-syn on human oligodendroglial differentiation. Most importantly, we aim to define molecular mediators by which human α-syn may interfere with oligodendroglial maturation and myelination and ultimately, test potential rescue strategies. Taken together, the proposed human cellular model of MSA will be an important tool to improve our understanding of the underlying molecular mechanisms of oligodendroglial α-synucleinopathy in humans and may define novel pro-myelinogenic targets for MSA therapy

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The role of the transcription factor TCF4 in cortical development

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The bHLH transcription factor TCF4 plays an important role in neurodevelopment as highlighted by its causal link to neurodevelopmental syndromes including intellectual disability and Pitt-Hopkins-Syndrome. The specific role of TCF4 in CNS development remains largely unknown. This project aims to define the function of TCF4 in cortical development through the analysis of TCF4 mutant mice. Histological analyses in TCF4 haploinsufficient and TCF4 KO mice revealed perturbations in the development of the caudal cortex at E14.5. At this developmental stage, the number of deep layer VI Tbr1+ neurons was increased and the proliferation of radial glia cells was decreased, Furthermore, haploinsufficient mice exhibit a partial loss of the caudal corpus callosum and an overall reduction of cortical thickness at P7. These datapoints could not be compiled for KO mice as they die perinatally. Currently, single cell RNA-Sequencing of cortical cells (E14.5) is conducted to gain insight how the haploinsufficiency or loss of Tcf4 affects the transcriptome, cellular pathways and cell identities of different subtypes of cortical cells.

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The impact of microRNA 204 on the oligodendroglial gene regulatory network

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Oligodendrocytes (OLs) are the myelinating cells of the central nervous system, physically shielding associated axons and allowing rapid signal conduction. For the complex maturation process of oligodendroglial precursor cells (OPCs) into mature OLs, Transcription factors (TFs) of the Sox protein family are of substantial importance. Especially Sox10 has been recently reported to drive the expression of microRNAs (miRs) in oligodendroglia. miRs are small 20-22 nt molecules of RNA, degrading or transcriptionally repressing target mRNAs via a multicomponent complex. Although at least 70% of all miRs are expressed in the central nervous system, only few have been shown to be of functional importance in OL maturation. We identified miR-204 as a novel Sox10 dependent miR affecting the oligodendroglial lineage. At first we analyzed screening data and validated putative candidates. Furthermore an interaction of miR-204 and Sox10 was confirmed in luciferase assays and electromobility shift assays. Subsequent transduction of miR-204 expressing retrovirus in primary rat OPCs limited proliferation and improved differentiation. These data indicate a functional role of miR-204 in oligodendrogenesis, thus contributing to a broader understanding of oligodendroglial maturation.

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Maternal high fructose diet programmed the astrocytic deficiency of glycolysis and oxidative phosphorylation

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Maternal intake with high fructose diet (HFD) during pregnancy and lactation impairs learning and memory of female offspring in adulthood. However, the underlying mechanisms are largely unknown. Astrocytes, a non-neuronal cell type in the brain, elevate lactate support in response to energy demand arising from high neuronal activity. We hypothesize that maternal HFD dampens the glucose metabolism of hippocampal astrocytes in offspring resulting in less lactate release. In this study, we dissected the profiles of glycolysis, oxidative phosphorylation (OXPHOS), insulin sensing and glucose uptake of hippocampal astrocytes obtained from female offspring of maternal regular diet (ND) and of maternal HFD. Results from Seahorse XF assays and enzyme activity assays indicated that the suppression of basal glycolysis and maximal glycolytic capacity, mitochondrial respiration and the activities of electron transport chain in HFD group. Moreover, the decrements of glucose transporter 1 (GLUT1) and glucose uptake in combination with the increments of insulin receptor A (IRA) and p85 subunit of phosphatidylinositide 3-kinases (PI3K) were detected in the HFD group. Pioglitazone, which is known to increase astrocytic glucose metabolism, effectively improved the suppressed glycolysis and OXPHOS with enhanced ATP content and lactate release. Moreover, the suppressions of GLUT1 and glucose uptake in HFD group were reversed to the ND level. Together, these results suggested that maternal HFD impairs the astrocytic glycolysis and OXPHOS. The metabolic deficiency, insulin signaling and glucose uptake were reversed by pioglitazone.

The transcription factor prospero homeobox protein 1 is a direct target of SoxC proteins during developmental vertebrate neurogenesis

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Early stages of neurogenesis require an intricate spatiotemporally controlled regulatory network. In addition to proneural genes, the high-mobility-group-domain containing SoxC transcription factors Sox4 and Sox11 and the homeodomain containing Prox1 have been associated with these processes. Both are expressed in neuronal precursors and neuroblasts and are required for the initiation of neuronal gene expression. Here, we report that Prox 1 is a direct target of SoxC proteins during spinal cord neurogenesis. By combining in vivo and in vitro binding studies with luciferase reporter assays, we find that SoxC proteins activate Prox1 expression through multiple regulatory elements by directly binding to them. These include the Prox1 promoter and two upstream evolutionary conserved enhancers at -44kb and -40kb relative to the transcription start. Furthermore, we show using electroporation in the chicken neural tube that Prox1 can activate only a subset of known SoxC target genes. Our data indicate that SoxC proteins and Prox1 share some functions, but also serve unique and non-overlapping tasks. SoxC-dependent regulation of Prox1 expression couples expression of both factors and may thereby ensure full induction of neuronal differentiation

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