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Introductory Course

IC01-02

Development of glial cells: changing from neurons to glia

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The development of the most complex biological organ, the Central Nervous System (CNS) comprising the brain and the spinal cord, is one of the most fascinating endeavors undertaken by evolution, and one of the most interesting topics to understand our own human complexity. Recent technological advances, including transcriptomic profiling of single cells, precise cell-lineage tracing, refined genetic tools for functional studies, and unprecedented imaging of the developing CNS, have given us a deeper inside into the variety of cell-types and the molecular mechanisms involved their generation, deployment, and homeostasis. In this course, we will look into the mechanisms involved in the generation of different neural cell-types focusing on glial cells, including the role of transcription regulators driving commitment toward oligodendroglia and astrocytes, the role of radial glia as the neural stem cells, and the mechanisms leading to the generation of different neural subtypes, such as cell-cell interaction, patterning, and timing. We will end up discussing how this knowledge is leading to optimise different protocols inducing neurogenesis and gliogenesis in the-dish, by reproducing normal development, or by shortcuts driving the generation of a neural subtype through reprogramming from one cell-type to another, with these ex-vivo models helping to unravel the specific changes proper to human CNS development during physiological and pathological conditions.

IC01-03

Astrocytes in health and diseases

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While it is well established that the neuron is the fundamental cellular unit of the brain, it is necessary to consider that it is not the only type of cells. Indeed, the human brain is composed of half neurons and half glial cells. Astrocytes, forming one class of glial cells are ideally positioned in between blood vessels and synapses. These glial cells have in addition the particularity to occupy exclusive territories, called “domains” in which each astrocyte is in close apposition with an exclusive number of synapses. Importantly, astrocytes are not isolated since they are connected to each other through gap junctions. At the end of the twentieth century, and since then, accumulation of data obtained in several laboratories around the world have indeed identified that astrocytes are involved in much more complex functions than initially expected, as they are able, among other functions to: i) uptake ions and neurotransmitters released during synaptic transmission, ii) detect synaptic transmission, iii) integrates synaptic information and in turn iv) release transmitters. The goal of this introductory course will be to give a general overview of the role of astrocytes from the cellular level to behavior in health and diseases.

IC01-04

Neuron-oligodendroglia interactions

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This introductory course focuses on different modes of communication between neurons and oligodendrocyte (OL) lineage cells that have been shown to be important in myelination and CNS function. These glial cells, whether oligodendrocyte precursor cells (OPCs) or OL, are capable of sensing their environment thanks to an array of receptors for neurotransmitters or external factors released by surrounding cells. Beyond this chemical communication, other types of neuron-oligodendroglia interactions take place in the brain, including those based on cell adhesion molecules or gap-junctions. We are still far from understanding the role of these multiple and complex neuron-glia interactions in the CNS. Nevertheless, recent studies show that these interactions have important implications on the myelination process as well as on the maturation and function of neuronal circuits. Moreover, alterations of neuron–oligodendroglia interactions may be at the origin of demyelinating disorders and other CNS pathologies. In this lecture, we will highlight some old and recent contributions that have improved our understanding of neuron-oligodendroglia interactions in health and disease. We will also discuss some issues that are currently unresolved and remain opened for future investigation.

Acknowledgement
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IC01-05

Myelin plasticity and regeneration

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Myelination is a late developmental event, with maximal activity after birth and reaching a plateau in young adulthood. Myelin sheathes are produced by oligodendrocytes that extend their plasma membrane and form compacted spirals around the axon. Myelin plays key roles in metabolic support to neurons and in signal conduction velocity and synchrony, therefore it is essential for proper brain functioning.
In the adult brain, myelin has long been considered as a fixed component. Yet, this last decade, numerous studies have unraveled the highly plastic nature of oligodendrocytes and myelin. Indeed, in the brain myelin is remodeled throughout life. Oligodendrocytes and their progenitors are sensitive to neuronal activity which drives myelination, and in turn myelin modifies neuronal circuit properties.

Spontaneous myelin regeneration can be observed in the adult brain. Following a demyelination insult, oligodendrocyte progenitors are mobilized: they proliferate and differentiate into myelinating oligodendrocytes, allowing cell replacement. Spared oligodendrocytes are also able to extend new myelin sheath thus contributing to myelin repair.
In this course, we will show how such plasticity has been discovered and examine its functional consequences both in physiological and pathological conditions.

IC02-03

Forgotten glial cells

A. Sharif

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This course aims at introducing non conventional glial cells that populate adult brain regions devoid of blood brain barrier, namely the circumventricular organs. The best known of them are tanycytes, which are found in the hypothalamus and man the dialogue between the brain and the periphery to regulate energy metabolism and reproduction. Neuroanatomical, molecular and functional aspects will be covered to illustrate the multiple strategies and the high level of plasticity used by tanycytes in the neuroendocrine brain. The role of tanycyte dysfunction in the development of metabolic disorders will also be discussed.

IC02-05

Insights into glioma development and cancer stem cells

E. Huillard

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Diffuse gliomas are tumors arising within the brain and constitute the most malignant primary brain tumors in adults. In this course, I will give an overview on gliomas, focusing on the most aggressive type, glioblastomas (GBM). I will discuss the mechanisms of GBM initiation and development from normal glial progenitors. I will introduce the notion of glioma stem cells and highlight their plasticity. I will describe the interactions between glioma cells and cells of the tumor microenvironment. Finally, I will discuss the emerging therapeutic options for these deadly tumors.

Plenary Lectures

L01-02

Astrocyte heterogeneity: from stem cells to repair

M. Götz

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Astrocytes are crucial for brain function buffering ions, regulating transmitters, synapse functions and much more throughout the CNS. Yet, they are also diverse – exhibiting differences between brain regions. In specific niches of the adult brain even have a very specialized function as neural stem cells which is performed in development by radial glial cells. I will address first the differences between astrocytes and neural stem cells and discuss some main molecular players as well as the importance of the local niche in instructing these stem cell properties. I will then discuss “intermediate” forms of parenchymal astrocytes with partial stem cell functions – in the intact, as well as injured brains. Finally, I will proceed to a new concept emerging from recent single cell RNA-seq data how astrocytes can perform both region-specific and brain-wide functions. If time permits I will discuss the relevance of these findings for brain repair.

L03-01

Oligodendrocytes and myelin: sculpting circuits in development and in adulthood

J. Chan

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Oligodendrocyte lineage cells are shaped by external experiences throughout life. Both passive experiences, like the availability of sensory stimuli, and active experiences, such as learning, can influence oligodendrocyte maturation as well as the pattern of myelin sheaths they produce. The relative stability of myelin, once formed, make it uniquely well suited to modulate long-term changes in neuronal circuit function. In this lecture, I will present recent work from my laboratory focusing on how oligodendrocytes and myelin shape neuronal circuits in the developing cortex and how adult oligodendrocyte/myelin plasticity interacts with neurons to support long-term memory.

Acknowledgement
This work belongs to Drs. Wendy Xin, Simon Pan and Feng Mei. We are grateful to our outstanding collaborators: Drs. Mazen Kheirbek, Michael Stryker, Megumi Kaneko, as well as the members of the Chan lab, past and present, for their valuable insight and support. This work was supported by the National Institutes of Health/National Institute of Neurological Disorders and Stroke (grants R01NS097428 & R01NS095889 to JRC and grant F32NS116214 to WX), the Adelson Medical Research Foundation (APND grant A130141 to JRC), and the Rachleff family endowment.

L04-01

Studying myelinated axon biology *in vivo* using zebrafish

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Myelinated axons are essential to nervous system formation, health and function. We use the zebrafish as a model organism to study myelinated axon biology, due to the suitability of zebrafish for detailed live imaging of cell behaviour, cell-cell interactions and neural circuit function in vivo over time, as well as their genetic conservation with mammals and experimental tractability, and their amenability for scalable drug screening, including in disease-relevant paradigms. In this plenary presentation I will provide an overview of ongoing projects that are investigating the growth in diameter of axons prior to myelination and mechanisms of myelination, particularly those of activity-regulated myelination. In addition I will describe our work studying the consequences of demyelination in vivo and our efforts to use zebrafish to identify potential therapeutics of relevance to promoting remyelination and neuroprotection. Finally, I will speak to the integration of studies in zebrafish with those in complementary mammalian models and human-based platforms.

L05-01

Astrocyte regulation of neuronal synapses

N. Allen

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I will present our work investigating how neuronal synapses are regulated throughout the lifespan. We approach this by asking how astrocytes regulate synapse number, synaptic function and synaptic stability. This includes identifying proteins secreted by developing astrocytes that are sufficient to induce immature synapses to form, as well as proteins produced by adult astrocytes that induce synapse maturation and limit synaptic plasticity. Further, I will present our work examining how astrocytes in the aging brain promote an environment conducive to synapse loss, and ask whether this contributes to progression of neurodegenerative disorders.

L06-01

Scar formation in the injured spinal cord – mechanisms and opportunities

J. Frisén

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Injuries to the central nervous system are inefficiently repaired and instead scar tissue forms at the lesion. We have investigated the origin and functional role of the scar tissue that forms after spinal cord injury in mice. Two very small cell populations, ependymal cells and type A pericytes, gives rise to the majority of new born scar forming cells. Spinal cord ependymal cells acquire neural stem cell properties after injury and gives rise to a large number astrocytes and few remyelinating oligodendrocytes. The ependymal cell reaction is required to regain the tissue integrity in the injured spinal cord. Type A pericytes gives rise to the fibrotic component of the scar, and they are necessary for closing the lesion. The identification of ependymal cells and pericytes as key components of scar formation after spinal cord injury allows manipulations of their response to lesions, with the aim to facilitate regeneration and functional recovery.

Symposia

S01 | Microglia shape white matter health

S01-01

Microglia refine myelin formation by phagocytosis during development

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During development, neurons and synapses frequently are formed in excess and then removed. Similarly, myelin sheaths sometimes are eliminated soon after they wrap axons. The frequency of myelin sheath removal is enhanced by silencing axons, suggesting that axon activity promotes sheath stability, but the mechanisms that mediate sheath removal have not been known. Because microglia phagocytose neurons and synapses, we hypothesized that microglia also phagocytose myelin sheaths to remove them from axons during development. We tested this hypothesis using live imaging to examine microglia and myelin in zebrafish larvae and genetic, pharmacological and behavioral approaches to manipulate neural activity and microglia function. We found that microglia phagocytosed individual myelin sheaths and not entire oligodendrocytes in the spinal cord and that phagocytosis accounted for most myelin sheath removal events in development. In the optic tectum, neuronal activity regulated how microglia associate with neuronal cell bodies and the amount of myelin they phagocytosed. Furthermore, zebrafish larvae depleted of microglia had excess and ectopic myelination. Altogether, our data indicate that microglia refine developmental myelination by sheath phagocytosis and that neuronal activity can influence the amount of myelin that microglia consume.

Acknowledgement
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S01-02

Microglial interaction with nodes of Ranvier in physiological and pathological contexts

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Myelination of axons ensures the rapid propagation of action potentials by saltatory conduction. This process relies on axonal segments insulated by myelin alternating with the nodes of Ranvier, short unmyelinated domains highly enriched in voltage-gated sodium channels (Na_V). In Multiple Sclerosis (MS), demyelination is associated with the disruption of the nodes of Ranvier (Craner et al, 2004; Coman et al, 2006), leading to a decreased conduction velocity or a failure of axonal conduction, which results in functional deficits. An endogenous repair process exists, which allows the restoration of myelinated fiber organization and of fast axonal conduction. This process is however partial and, with time, neurodegeneration can occur. Better understanding how to promote remyelination and neuroprotection in MS is thus essential.

It has been shown that nodal reclustering is an early event during this process and could thus participate in repair (Coman et al, 2006). Microglia, the resident immune cells of the central nervous system, are also key players in MS repair processes, as they are a major modulator of remyelination (Lloyd et al., 2019; Miron et al., 2013).

We identified the nodes of Ranvier as a direct and preferential site of interaction between microglia and axons, in both mouse and human tissues. We show that microglia-node interaction is modulated by neuronal activity and stabilized by axonal potassium release. Disrupting axonal potassium release or its read-out by microglia following demyelination decreases microglial polarization towards its pro-regenerative state and impairs remyelination.

Taken together, these findings identify the node of Ranvier as a major site for communication between neurons and microglia, which could participate in homeostasis and repair.

S01-03

Microglia regulate the integrity of myelin in the central nervous system

V. Miron

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Efficient functioning of the central nervous system (CNS) requires myelin, formed by oligodendrocytes that ensheath neuronal axons with tightly compacted layers of membrane of an appropriate thickness. Disrupted CNS function with ageing or neurodegenerative disease is associated with changes in myelin structure, such that it is made in excess and unravelling, less compacted, thicker, and eventually degenerates. However, the mechanisms instructing the appropriate formation and integrity of myelin are unclear. Recent work has implicated CNS-resident macrophages termed microglia in supporting oligodendrogenesis and myelination, based on impairments in these processes following microglial depletion via loss-of-function of the pro-survival colony stimulating factor (CSF)-1 receptor. As this approach also targets other CNS macrophages (such as perivascular macrophages) which may contribute to these processes, the specific contributions of microglia to myelin formation and integrity are unknown. To address this, we utilised a recently developed transgenic model in which deletion of the FIRE super-enhancer of the *Csf1r* gene (Csf1r-FIRE$^{\Delta/\Delta}$) leads to an absence of microglia from development (when they normally emerge) through to adulthood, while retaining other CNS macrophages. We found that in the absence of microglia in Csf1r-FIRE$^{\Delta/\Delta}$ mice, oligodendrocytes and myelin were still formed in the white matter. However, Csf1r-FIRE$^{\Delta/\Delta}$ mice showed a loss of integrity of myelin, with i) increased outfoldings and unravelling, ii) impaired compaction, iii) increased thickness, and iv) eventual demyelination. We discovered similar loss of myelin integrity in a human

condition (ALSP) whereby heterozygous mutations in CSF1R results in reduced white matter microglia. We reveal that microglia regulate developmental myelination at later stages than previously thought, supporting the integrity of myelin once it is formed, rather than driving the generation of oligodendrocytes and initial myelin ensheathment. We uncovered that microglia are required for myelin structural integrity and maintenance. Our findings demonstrate the critical role of microglia in supporting myelin integrity, and have important implications for understanding the pathological mechanisms underpinning the loss of myelin integrity in ageing and neurodegenerative diseases.

S01-04

Single cell mass cytometry of human white matter microglia in health and disease

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Myeloid cells contribute to inflammation and demyelination in multiple sclerosis (MS). Here, we haveharnessed the power of single-cell mass cytometry (CyTOF) with up to 74 targeted proteins to studymyeloid cell phenotypes in normal-appearing white matter and in active lesions of progressive MS. Wedetected significant differences between microglia from white matter and grey matter in the human brain.In active MS lesions, CyTOF measurements revealed a decreased abundance of homeostatic and TNF^{hi} microglia, and an increase in highly phagocytic and activated microglia states. In contrast to studiesof the early inflammatory disease stages of MS, infiltrating monocyte-derived macrophages were scarce inactive lesions of progressive MS, suggesting fundamental differences of myeloid cell composition inadvanced stages of MS.

Acknowledgement
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S02 | Transcriptional control of reactive astrocyte responses across neurological diseases

S02-01

Developmental insight into diverse reactive astrocyte responses after injury and degeneration

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Astrocytes are the most abundant type of glial cell in the CNS and play vital roles in all facets of brain physiology, ranging from neurotransmission, synaptogenesis, and circuit function, to metabolic support and formation of the blood brain barrier. Moreover, astrocyte function is altered in response to injury, degeneration, and aging, illustrating an additional layer of cellular plasticity that likely contributes to their diversity. We recently developed an intersectional, FACS-based strategy that identified five molecularly and functionally distinct astrocyte subpopulations in the brain. One of these subpopulations is specifically labeled by the cell surface maker CD51 and is endowed with enhanced synaptogenic function. These synaptogenic functions led to examine the role of CD51+ astrocytes in functioning brain circuits. To determine how CD51+ astrocytes contribute to circuit function, we created new mouse lines that enable us to use intersectional genetics to selectively label and manipulate this population of astrocytes in the native brain. To decipher the mechanisms that regulate CD51+ astrocytes, we utilized our molecular profiling data and identified key developmental transcription factors are enriched in this subpopulation and exhibit dynamic expression in the aging brain and in degenerative models. Preliminary studies with newly generated mouse line that specifically eliminate these core transcription factors from CD51+ astrocytes revealed selective regulation of astrocyte morphology and circuit function in the hippocampus and olfactory bulb in an aging-dependent manner.

S02-02

Role of reactive astrogliosis in Alzheimer's disease and stroke

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The molecular heterogeneity of reactive astrocytes within and across different diseases, as well as their beneficial or detrimental impact on neuronal survival, remain incompletely understood. This talk will focus on the role of

reactive astrocytes in two brain pathologies – acute ischemic stroke and Alzheimer's disease (AD). In animal models of stroke, we will present data that the dysfunction of ion and glutamate transporters in astrocytes contributes to calcium elevations in neurons and astrocytes as well as to extracellular glutamate accumulation. Consequently, transgenic or pharmacological interference with these pathways attenuates synaptic disequilibrium and improves outcome. In the chronic phase of ischemia, transcriptome analyses show a considerable molecular heterogeneity of reactive astrocytes within the glial scar, and targeting subpopulations of astrocytes by genetic or pharmacological manipulation can improve neuronal survival and axonal recovery after stroke. In AD models, we will show that an imbalance of astroglial calcium signaling, in part induced by pro-inflammatory activation of near-plaque subpopulations of reactive astrocytes, contributes to neuronal-glial network disequilibrium and cognitive dysfunction. Moreover, these network changes as well as pro-inflammatory glial signaling cascades are also reflected by transcriptional changes of reactive astrocytes in AD models, and manipulating these pathways attenuates network dysfunction, synaptic loss and cognitive decline. Hence, functional and molecular characterization and manipulation of disease-specific aspects of reactive astrogliosis may guide the development of astrocyte-targeting therapies.

Acknowledgement

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S02-03

Transcriptional regulation of astrocyte plasticity in brain homeostasis, injury and tumor-associated astrocytes

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Astrocytes perform vital functions in the central nervous system (CNS), contributing to metabolic support, synaptic function and synaptogenesis, endocrine signalling, blood-brain-barrier maintenance, as well as injury response and repair. Developmentally related to radial glia and neural stem cells, astrocytes retain a remarkable level of cell plasticity that is most apparent in their regenerative capacity in lower vertebrates. Even in mammals, reactive astrocytes partially adopt stem cell states, although this process is blunted. How astrocyte plasticity is regulated at the transcriptional level remains incompletely understood. Epithelial-mesenchymal transition (EMT) is a cellular program that enables cells to acquire stem cell characteristics and increase their motility. EMT is an essential step during development and wound repair, as well as in certain disease processes. Fundamentally, EMT is a stem cell program, and several EMT transcription factors (e.g. members of the SNAI or ZEB families) are regulation key stem cell functions. Whether these transcription factors regulate cell plasticity in the CNS is not fully understood. We have previously found that the transcription factor ZEB1 is a master regulator of stemness in glioblastoma, the most common type of adult brain cancer. Recent studies have shown that ZEB1 also acts during embryonic development, where it regulates self-renewal and lineage selection in the CNS. Using new transgenic murine models for conditional-inducible deletion of Zeb1 in neural stem cells and astrocytes, we evaluate the functions of ZEB1 in the adult brain. Loss of Zeb1 causes differentiation of neural stem cells in the adult hippocampus, resulting in a temporary increase of neurogenesis, which is accompanied by a reduction of astrocyte numbers. In cortical stab lesion injury, we use conditional Zeb1 deletion to evaluate the how cell plastricity contributes to reactive astrogliosis and glial scarring. Likewise, deletion of Zeb1 in tumor-associated astrocytes allows evaluating the role of astrocyte cell plasticity in the glioma microenvironment for tumor progression and invasion.

S02-04

Systematic analysis of astrocytes after spinal cord injury unveils heterogeneity and important regulatory genes in astrogliosis

J. Wu

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To better understand the functions and interactions of the cell types in the brain, we have previously purified representative populations of neurons, astrocytes, oligodendrocyte precursor cells, newly formed oligodendrocytes, myelinating oligodendrocytes, microglia, endothelial cells, and pericytes from mouse cerebral cortex, collaborating with Dr. Ben Barres group. We generated a transcriptome database for these cell types by RNA sequencing. Recently, we have comprehensively investigated the molecular changes in the injury environment and the astrocyte-specific responses by astrocyte purification from injured adult spinal cords from acute to chronic stages. In addition to protein-coding genes, we have systematically analyzed the expression profiles of long non-coding RNAs (lncRNAs) (>200 bp), which are regulatory RNAs that play important roles in the CNS. Bioinformatic and functional analyses identified a highly conserved lncRNA *Zeb2os*, and we demonstrated it plays an essential role in reactive astrogliosis through the *Zeb2os/Zeb2/Stat3* axis. Viral mediated knockdown of *Zeb2os* in subacute stage of spinal cord injury (SCI) led to reduced astrogliosis, lesion size and pSTAT3 in injured animal models (PMID: 33535036). Overall, these studies provide valuable insights into the molecular basis of reactive astrogliosis and fill the knowledge gap regarding the functions of lncRNA in astrogliosis and SCI.

Currently, my group is dissecting molecular and cellular constituents of reactive astrocytes in neurological injury and disorders using single cell sequencing, advanced comparative bioinformatics and functional tests. Studies from us and others indicate that astrocytes are highly heterogeneous in gene profiles and morphology (PMID: 33589835). How diverse constituents of astrocytes contribute to neurological disorders remains elusive. Understanding reactive astrocyte states and roles can open new avenues for the development of improved therapeutics.

S03 | Microglial immunometabolism: relevance in normal and disease states

S03-02

Role of lipid nutrients on microglial function in the developing brain

A. Nadjar

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My research focuses on the nutrient sensing by microglia in various pathophysiological contexts. Here, I will discuss a new mechanism by which low maternal omega-3 intake alters the shaping of neural networks during neurodevelopment by disrupting microglia-mediated synaptic pruning in the hippocampus. Our data show that maternal dietary n-3 PUFA deficiency increases microglial phagocytosis of synaptic elements in the developing hippocampus, through the activation of 12/15- lipoxygenase (LOX)/12-HETE signaling, which alters neuronal morphology and affects cognition in the postnatal offspring. These findings provide new insights into the mechanisms linking maternal nutrition to neurodevelopmental disorders.

S03-03

Targeting Mitochondrial Metabolism in Neuroinflammation with Neural Stem Cells and their Cargo Vesicles

S. Pluchino

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Advances in stem cell biology have raised great expectations that diseases of the central nervous system may be ameliorated by the development of non-haematopoietic stem cell medicines. Yet, the application of stem cells as therapeutics is challenging and the interpretation of some of the outcomes ambiguous. The initial idea that stem cell transplants work only via structural cell replacement has been challenged by the observation of consistent intercellular information exchange between the graft and the host. Sustained stem cell graft-to-host exchange of signals has led to remarkable trophic effects on endogenous brain cells and beneficial modulatory actions on innate and adaptive immune responses that ultimately promote the healing of the injured CNS. Among a number of promising candidate stem cell sources, neural stem/precursor cells (NSCs) are being extensively investigated for their capacities to *signal* to the immune system upon transplantation in experimental CNS diseases.

Here I will discuss a new mechanism of cellular licensing by which transplanted and endogenous NSCs counteract CNS-compartmentalised chronic inflammation in mice.

I will also focus on defining whether the form of cellular signaling mediated by extracellular membrane vesicles (EVs) exists for NSCs, and on its molecular signature and functional relevance on target cells.

I will then share evidence that the EV cargo molecules are modulated by extracellular pro- or anti-inflammatory cytokines and determine the key elements responsible for this novel mechanism of EV-mediated intercellular communication.

I will finally reflect on the forthcoming challenges related to the translation of some of these exciting experimental proofs into ready-to-use clinical medicines for inflammatory CNS diseases.

S03-04

Hypothalamic microglia-neuron interaction in obesity and type 2 diabetes.

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Brain microglia are long-surviving and self-renewing innate immune cells that are crucial for scavenging cell debris and pathogens to maintain brain tissue homeostasis. The hypothalamus contains highly heterogeneous and condensed populations of neurons in different regions that control whole body energy metabolism. It is reasonable that this region constantly produces cell debris and metabolic waste during different metabolic states. In order to keep a healthy and clean microenvironment for the hypothalamic neurons to function, the microglial activity in the hypothalamus needs to match the high demands for immune surveillances and debris phagocytosing/clearances. This was demonstrated by the fact that microglia in the mediobasal hypothalamus showed a significantly higher reactivity than in other regions when experimental animals were exposed to high-fat high-sugar (HFHS) diet, and that this occurs rapidly after receiving the HFHS diets. The reactive microglia in the mediobasal hypothalamus upon HFHS diet is not only characterized by increased cytokine production, but also impaired phagocytic capacity, as shown by a downregulation of phagocytic indicator CD68 expression in HFHS diet-fed rats. In microglia, besides governing lipid uptake for fueling, the lipoprotein lipase (LPL)-gated phospholipid production is also crucial for phagolysosome formation and turnover. In HFHS diet-fed mice that has lack of LPL in microglia, there was a worsened phagocytic capacity, ultimately associated with lesser anorexigenic pro-opiomelanocortin (POMC) neurons and more vulnerability to diet-induced metabolic disorders. Thus, lacking a sufficient microglial phagocytosis has a detrimental effect on POMC neural survival upon diet challenge. Under physiological conditions, the neural activity in the hypothalamus varies during day-night cycle, hypothalamic microglial cells also exert their function in a strict time-of-day manner with higher activity during the dark, active phase, compared to the light, sleep phase. However, so far, it is not clear how this intrinsic clock relates to the microglial immune and phagocytic function, especially in stimulated conditions such as in the control of systemic energy homeostasis. We

generated mice with microglia-specific knock-down of the core clock gene, *Bmal1*. Interestingly, we found an increased microglial phagocytosis in mice subjected to HFHS diet-induced metabolic stress. This enhanced microglial phagocytosis was associated with significant retention of POMCexpression in the mediobasal hypothalamus, and significantly less body weight gain upon HFHS diet. We conclude that loss of the rigorous control implemented by the intrinsic clock machinery increases the extent to which microglial phagocytosis can be triggered by neighboring neurons under metabolic stress during any time of the day. Ultimately, this ensures a healthier microenvironment in the hypothalamus for the neighboring metabolism-regulatory neurons to function and protects animals from diet-induced obesity.

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S05 | Mechanisms and effects of endocannabinoid signaling in glial cells

S05-01

Functional consequences of endocannabinoid signaling at Tripartite Synapses

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The endocannabinoid (eCB) system is an important intercellular signaling system involved in a wide variety of physiological processes. In the brain, eCB signaling regulates multiple biological functions such as pain perception, food intake, learning and memory, anxiety and cognition. While the canonical mechanism of eCB signaling is the activation of CB1 receptors in neurons and the consequent regulation of synaptic transmission and plasticity, accumulating evidence is revealing novel and important roles of eCB signaling in brain function through the actiatio of CB1 receptors in astrocytes.

The experimental results recently obtained in our lab regarding the mechanisms and functional consequences at synaptic, circuit and behavioral levels of the astrocyte activation by endocannabinoids (eCBs) will be presented and discussed. Our results on eCB-mediated astrocyte-neuron signaling in different brain areas, including striatum, hippocampus, cortex and amygdala, shows: 1) the existence of functional astroglial-neuronal networks comprising subpopulations of astrocytes, neurons, and synapses; 2) the synapse-specific astroglial control of synaptic function in these brain areas; 3) the neuronal activity-dependent ability of single astrocytes to release distinct gliotransmitters; 4) the contribution of astrocytes to hippocampal synaptic plasticity; 5) the astrocyte-mediated lateral synaptic regulation in the somatosensory cortex; and 6) the layer-and column specific eCB signaling in the cortex. Finally, I will also present recent evidence showing the behavioral consequences of the eCB signaling to astrocytes in hippocampus- and amygdala-associated animal behaviors.

Acknowledgement

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S05-02

Cortical cannabinoid receptor type-2 signalling in microglia-to-neuron communication

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Increasing evidence points to identify the endocannabinoid (eCB) system among signallings regulating microglia-neuron communication. Microglia express components of the eCB system, including receptors, ligands, and metabolic enzymes. In particular, the cannabinoid (CB) type 2 receptor (CB2R) is strongly regulated in microglia during several disease states. In particular, the selective stimulation of CB2R suppresses microglial activation and promotes a microglial anti-inflammatory phenotype. However, direct evidence on the functional expression of CB2R in microglia is still missing. To overcome this issue, we provide evidence on the functional expression of this receptor in cortical microglia by measuring microglia CB2-activated currents. Noticeably, microglial CB2R stimulation, selectively modulates neurotransmission at glutamatergic synapses, while it does not affect GABAergic transmission. Moreover, we observed that microglia-neuron CB2R signalling is altered in the cortex of mice suffering from chronic pain. In the end, we propose the CB2R signalling as a new route for microglia –neuron communication

S05-03

Mitochondrial CB1 receptors: beyond the "metabolic" and "synaptic" dychotomy of astroglial functions

G. Marsicano

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Astrocytes control brain functions through different mechanisms, and (endo)cannabinoid signaling seems to regulate many of them. Beyond the usual dychotomy between "synaptic" and "metabolic" functions, recent data indicate that these different astroglial functions converge to regulate information processing in the brain. In this talk, I will present different aspects of (endo)cannabinoid control of astroglial functions and their impact on brain signaling and behavior. In particular, I will describe how type-1 cannabinoid present at astrocyte mitochondria (mtCB1) can at the same time control astrocyte glucose processing and determine the dynamics of intracellular calcium signaling. Thus, astroglial mtCB1 receptors regulate both "metabolic" (glucose) and "synaptic" signaling (calcium), resulting into alterations of brain functions and behaviour.

S05-04

Endocannabinoid-mediated control of astrocytes in multiple sclerosis

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Demyelinating lesions of multiple sclerosis (MS) patients show dysfunctional astrocytes that exhibit a neurotoxic profile *in vitro*. The endocannabinoid system has the potential to attenuate neurodegeneration and relieve symtomatology in MS but the precise mechanisms of cannabinoid action during autoimmune demyelination are far from being understood. Astrocytes are endowed with cannabinoid CB_1 receptors that modulate synaptic communication and celular metabolism with potential implications in neuroinflammatory disease contexts. Nonetheless, the possibility that astroglial cells represent important targets and mediators of endocannabinoid signaling in MS has been disregarded for a long time.

I will present data on how endocannabinoids modulate astrocytic functions in MS using RT-qPCR, FACS, immunohistochemistry and experimental autoimmune encephalomyelitis (EAE) to model the disease. Astrocytes purified during EAE exhibit an early-onset deregulation of endocannabinoid signaling-associated genes with decreased expression of hydrolyzing enzymes (*Mgll*, *Abhd6*) and increased levels of the synthetic enzyme *Napepld*. These transcriptional adaptations precede the induction of neurotoxic phenotype genes thus suggesting that astrocytic CB_1 receptors may modulate the acquisition of pathogenic functions by these cells during EAE. Conditional mutant mice lacking CB_1 receptors in astrocytes display significantly decreased EAE clinical scores paralleled by reduced lesion load, T cell infiltration, microglial and astroglial reactivity and neuronal damage. In the end, we propose that endocannabinoid signaling through astrocytic CB_1 receptors contribute to the pathogenic activation of these cells during neuroinflammation and thus counteract the general protective function of the endocannabinoid system in MS. A further exploration of the underlying mechanisms will help decipher the mechanisms driving astrocyte dysfunction in MS thus paving the way for the development of more efficacious therapeutic approaches.

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S06 | Myelin dynamics in neurological function and dysfunction

S06-01

Oligodendrogenesis and Myelin Formation in Memory Consolidation

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The generation of myelin-forming oligodendrocytes persists throughout life and is regulated by neural activity. It is hypothesized that experience-dependent myelination contributes to neural circuit modification and memory consolidation. We will review data showing water maze learning and fear conditioning promotes oligodendrogenesis and myelin formation. Preventing these learning-induced increases in oligodendrogenesis without affecting existing oligodendrocytes impairs memory. These results suggest that de novo myelination tunes activated circuits, promoting coordinated activity that is important for memory consolidation. Consistent with this, contextual fear learning increased the coupling of electrophysiological signals between the hippocampus and cortex, and when oligodendrogenesis was suppressed no increase in signal coupling was observed. These data identify a non-neuronal form of plasticity that remodels circuits following learning and support memory consolidation.

S06-02

Glia-mediated Mechanisms of Chemotherapy-related Cognitive Impairment

E. M. Gibson

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Chemotherapy results in a frequent yet poorly understood syndrome of long-term neurological deficiencies. Neural precursor cell and white matter dysfunction are thought to contribute to this debilitating syndrome. Here, we demonstrate persistent depletion of oligodendrocyte lineage cells in humans who received chemotherapy. Developing a mouse model of methotrexate chemotherapy-induced neurological dysfunction, we find a similar depletion of white matter OPCs, increased but incomplete OPC differentiation and a persistent deficit in myelination. OPCs from chemotherapy-naïve mice similarly exhibit increased differentiation when transplanted into the microenvironment of previously methotrexate-exposed brains, indicating an underlying microenvironmental perturbation. Methotrexate results in persistent activation of microglia and subsequent astrocyte activation that is dependent upon inflammatory microglia. Microglial depletion normalizes oligodendroglial lineage dynamics, myelin microstructure and dynamics, and cognitive behavior after methotrexate chemotherapy. These findings indicate

that methotrexate chemotherapy exposure is associated with persistent tri-glial dysregulation and identify inflammatory microglia as a therapeutic target to abrogate chemotherapy-induced neurological dysfunction. These therapeutic strategies will depend on understanding microglial population dynamics. Recent work from our lab has identified a temporal susceptibility of microglial reactivity to chemotherapy toxicity, suggesting the potential for a chronotherapeutic approach to mitigate the tri-glial dysregulation associated with chemotherapy-related cognitive impairment.

S06-03

Social experience-dependent myelin plasticity: an epigenetic view

J. Liu

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Emerging evidence shows that oligodendrogenesis and myelination are highly responsive to behavioral experience, including physical activity and social experience. Transcriptional, translational and ultrastructural changes in the oligodendrocyte and subsequent myelin have been identified and represent powerful mechanisms to regulate neuronal circuit function. Our laboratory has demonstrated that various traumatic social experience has a significant impact on myelination and oligodendrocyte, the myelin-forming cells. Impaired myelination preceded depressive-like behavior in socially isolated mice and was associated with aberrant epigenetic modifications in the oligodendrocytes. Here we identified region-specific myelination differences in mice that displayed distinct behavioral outcomes in response to the same social stress. Myelin deficits were detected only in the medial prefrontal cortex (mPFC) of mice displaying social avoidance behavior ("susceptible"), but not in those who escaped the deleterious effect to stress ("resilient"). Furthermore, fewer mature oligodendrocytes and decreased heterochromatic histone marks were observed in mPFC of susceptible mice, highlighting the region-specific dynamic response of myelination as critical determinant of the avoidance response to traumatic social experiences. Finally, we explored the functional consequence of restoring myelination in relieving social behavior deficits in rodents and the underlying epigenetic mechanisms in oligodendrocyte.

S06-04

Myelin influences synaptic plasticity in the adult mouse cortex

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Developmental and adult myelination modulate action potential conduction velocity in the central nervous system (CNS) to regulate the function of neural circuits relevant to movement and memory. To determine what impact existing and new myelin exerts on synapse number, maturity or plasticity in adulthood, we prevented the addition of

new myelin (*Pdgfrα-CreER^{T2} ::Rosa26-YFP :: Myrf $^{fl/fl}$* or OPC-*Myrf*-deleted mice) or compromised the function of existing myelin (*Pdgfrα-CreER^{T2} ::Rosa26-YFP :: Myrf $^{fl/fl}$* or OL-*Myrf*-deleted mice) in the adult mouse CNS. The conditional deletion of *myelin regulatory factor* (*Myrf*) from oligodendrocyte progenitor cells (OPCs) reduced oligodendrogenesis by >80% in the adult mouse motor cortex and corpus callosum. Within 60 days, this impaired fine motor coordination, but did not impact callosal compound action potential (CAP) conduction velocity; gross motor function; the ability of mice to perform tasks that relied on intact short-term or working memory, or their ability to undertake spatial and reward-based learning. However, after 345 days, fewer axons were myelinated in the corpus callosum of OPC-*Myrf*-deleted mice compared with age-matched controls. By contrast, OL-*Myrf*-deleted mice developed gross motor dysfunction and CAP velocity was reduced by >39% within 35 days. We next crossed these mice with *Thy1-YFPH* mice to evaluate the impact of myelin loss on synaptic plasticity in the motor cortex. We found that OL-*Myrf*-deleted Layer V pyramidal neurons receive an equivalent number of inhibitor inputs (miPSCs) as those in the control motor cortex, and iPSC amplitude, rise and decay times suggest that the number and composition of GABA(A) receptors is also unchanged. Total spine density was also unchanged, but a higher proportion of the spines were mature, mushroom spines in OL-*Myrf*-deleted mice compared with controls, suggesting that myelin influences synaptic plasticity in adulthood.

S07 | Molecular mechanisms controlling neural stem cell dynamics during aging

S07-01

Mathematical modeling of age-dependence of adult neurogenesis

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The talk is devoted to mathematical modelling of adult neurogenesis. We propose mechanistic models of neural stem cell (NSC) activation, self-renewal and differentiation that allow quantifying and interpreting experimental data and provide new insights into the structure and dynamics of adult neurogenesis in the hippocampus and in SVZ. Integration of the mathematical approach with experimental data suggests that age-related changes of neural stem cell numbers and their activity can be explained by age-dependent down-regulation of the rate of release from quiescence (activation). Reduced activation prevents depletion of the stem cell pool. Blocking this mechanism in IFN knockout indicates a compensation ability of the system that prevents the loss of stem cells through an increase of self-renewal. Applying our models to data including counts of dormant and resting NSC (NSC that never divided versus those that did divide during post-embryonic development, respectively), suggests that activation rates of resting NSC are higher compared to those of dormant HSC. This implies that history of divisions contributes to stem cell heterogeneity. In this talk I will discuss challenges and limitations of mathematical modelling in the context of experimental data of adult neurogenesis.

S07-02

Glucocorticoid circadian oscillations control the architecture of the adult hippocampal neurogenic niche in the aging brain.

C. P. Fitzsimons

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A reduction in adult hippocampal neurogenesis has been linked to age-related cognitive impairment. However, the mechanisms involved in this age-related reduction remain elusive. Glucocorticoid hormones (GC) are important regulators of neural stem/precursor cells (NSPC) proliferation and other relevant cell types present in the adult hippocampal neurogenic niche. We have demonstrated before that GC and their physiological circadian oscillations are crucial for the functional integration of immature neurons in the dentate gyrus[1]; modulate age-associated changes in microglial morphology in the dentate gyrus[2], and preserve specific populations of adult hippocampal neural stem cells in the aging dentate gyrus[3]. These results indicate that the circadian oscillations in GC levels control the adult hippocampal neurogenic niche architecture in the aging brain.

In this lecture, I will discuss our results, put them in context with several other key studies in the literature and propose an integrative explanation for the role of GC as regulators of different glial cell types present in the adult hippocampal neurogenic niche during aging, including microglia, astrocytes, and neural stem cells.
Our results have translational relevance since disruptions in GC circadian oscillations are observed in prevalent age-associated, stress-related disorders and depression, and in aged patients chronically treated with synthetic anti-inflammatory GC, indicating that the cognitive impairment associated with these conditions may result from substantial changes in the architecture of the adult hippocampal neurogenic niche.

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S07-04

Origin and Aging of Adult Hippocampal Neural Stem Sells

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In the dentate gyrus (DG) of the hippocampus of most mammals, a population of radial glia-like quiescent neural stem cells (NSCs) with astrocytic properties generates neurons and astrocytes throughout adulthood. Newborn neurons participate in the new memory formation; learning; and responses to stress, anxiety, and fear. Adult neurogenesis, however, declines sharply with age mostly because of the progressive depletion of NSCs linked to their activation to generate precursor cells (Encinas et al. 2011).
The initial size of the adult population of NSCs is the main determinant for the total neurogenic output of the hippocampus and any alterations affecting the origin, molecular cues and critical time window of the generation of adult NSCs might provoke a life-lasting detrimental effect on neurogenesis. We have now stablished that adult NSCs are generated through cycline D2-dependent mitosis on-site in the DG during a critical early postnatal period and their properties (mitotic rate, biomarker expression) are different from developmental NSCs. Consequently, adult neurogenesis should not be considered a mere continuation of developmental neurogenesis.
Adult NSCs characterize by their neurogenic asymmetric division, and although self-renewal and symmetric division are possible, in the young brain there is a strong imbalance in favor of the activation-dependent depletion of neural stem cells (NSCs) that would lead to their rapid exhaustion (Martín-Suárez and Encinas 2021). Yet, in older brains, unexpected NSCs and neurogenesis remain. By slowing down overall NSC activation, their depletion is decelerated (Martín-Suárez et al. 2019). The slowdown occurs because several subpopulations of NSCs with

diverging behavior remain in the DG. One of them becomes more and more quiescent (omega-NSCs), thanks in part to the progressive inflammation associated to aging. The other remains “young” and maintains the capability to get activated (alpha-cells) (Martín-Suárez et al. 2019). Thus, paradoxically, adult neurogenesis remain for longer in the mammal brain at the expense of reducing neurogenesis.

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S08 | Blood brain barrier, neuronal activity and glia

S08-01

Neurovascular Interactions: Mechanisms, Imaging, Therapeutics

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The neurovascular interface fundamentally changes during disease due to increased blood-brain barrier (BBB) permeability and influx of blood proteins in the CNS parenchyma. The blood coagulation protein fibrinogen is deposited in the brain in a wide range of neurological diseases and traumatic injuries with BBB disruption. In my laboratory we uncovered pleiotropic roles for fibrinogen in neuroinflammation, neurodegeneration, and inhibition of remyelination[1,2]. Furthermore, we developed novel methods for imaging BBB disruption at the neurovascular interface with high-resolution in vivo two-photon microscopy and 3D volume imaging and identified fibrinogen as a new culprit for microglia-mediated spine elimination[3]. We discovered an unanticipated role for microglia dynamics in preventing neuronal network hyperexcitability[4]. Our research on transcriptional profiling of oxidative stress (Tox-Seq) identified neurotoxic CNS innate immune cell populations and therapeutic targets in neuroinflammation[5]. We developed fibrin-targeting immunotherapy to selectively target proinflammatory functions of fibrin without interference with effects on clotting[6]. Fibrin-targeting immunotherapy inhibits autoimmunity- and amyloid-driven neurotoxicity in animal models of multiple sclerosis and Alzheimer's disease, suggesting selective fibrin targeting might be beneficial for suppressing vascular-driven neurodegeneration[6].These findings could be a common thread for the understanding of the etiology, mechanisms of progression, and the development of new treatments for several neurologic diseases with cerebrovascular alterations and deposition of fibrin in the CNS.

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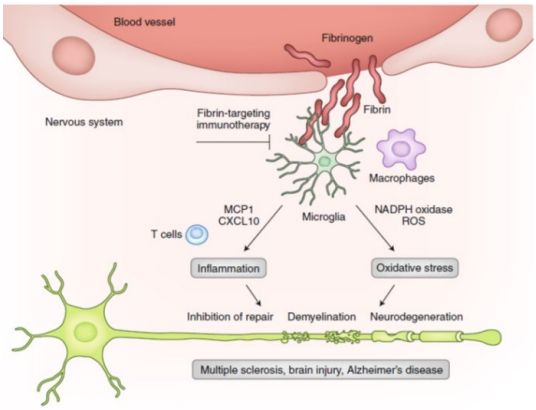

Mechanisms Linking BBB disruption with Neuroinflammation

S08-02

Astrocyte-derived Wnt growth factors are required for endothelial blood-brain barrier maintenance

S. Liebner

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Endothelial Wnt/β-catenin signaling regulates blood-brain barrier (BBB) differentiation and maintenance. In the adult brain both, pericytes (PCs) and astrocytes (ACs) participate in BBB integrity however, their contribution to Wnt/β-catenin signaling is poorly understood. By conditionally knocking out the *evenness interrupted* (Evi) gene in astrocytes of GFAP-Cre:Evi$^{lox/lox}$ mice (Evi$^{\Delta AC}$), we abolished Wnt factor release by ACs and analysed the effects on BBB maintenance.

Evi$^{\Delta AC}$ mice developed brain edema and increased vascular tracer leakage. While brain vascularization and endothelial junctions were not altered in 10 and 40 week-old mice, endothelial caveolin1(Cav)-mediated vesicular transport was increased *in vivo* and *in vitro*. Moreover, astrocytic end-feet were swollen and aquaporin-4 distribution was disturbed, coinciding with decreased astrocytic Wnt activity. Vascular permeability correlated with increased neuronal activation by c-fos staining, indicative of altered neuronal function. Astrocyte-derived Wnts thus serve to maintain Wnt/β-catenin activity in endothelia and in astrocytes, thereby controlling Cav-1 expression, vesicular abundance, and end-feet integrity at the NVU.

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S08-03

Blood-brain barrier pericytes reactivity and MRI in epilepsy: from pathophysiology to target strategies

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Blood-brain barrier damage promotes and sustains abnormal neuronal discharges. This direct link is contingent to endothelial permeability, pericytes damage and reactivity, astrogliosis, and formations of perivascular inflammatory scarring. Here, we outline salient findings showing how pericytes are involved in the pathophysiology of the neurovascular unit during seizure disorders, in humans and experimental models. Next, we propose an innovative diagnostic MRI strategy that exploits neuro-glio-vascular damage as an entry point to define pathological seizure networks.

S08-04

The impact of enhanced neuronal activity on neuron-OPC interactions in the hippocampus

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NG2 cells, or oligodendrocyte progenitor cells, are a glial cell population that covers the entire parenchyma of the central nervous system. NG2 cells differentiate into mature oligodendrocytes, ensheathing axons with myelin. However, it is still unknown whether all NG2 cells have the same potential to generate oligodendrocytes or whether a subpopulation of them becomes permanent NG2 cells. The mechanisms of neuron-NG2 cell interaction are not fully understood, but this interaction is thought to be regulated by neuronal activity. In addition, it is currently debated whether NG2 cells interact with other cells types of the central nervous system, or maybe even part of the neurovascular unit. Using a juvenile genetic mouse model for epilepsy, we investigate how altered neuronal excitability affects the structural relationship between neurons and NG2 cells in specific layers of the hippocampus. We will discuss whether alterations in morphology have a functional implication. With transcriptome analysis and chromatin accessibility studies, we assess the difference in gene expression in NG2 cells upon increase in neuronal activity. Finally, we describe the spatial relationship between NG2 cells and the vasculature and discuss the possible functional role these interactions will have.

Acknowledgement

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S09 | Glial cells in the limelight of metabolic signaling: experiments in mouse and fly

S09-01

Astroglial insights to the paradox of brain aerobic glycolysis

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The Barros Lab uses genetically-encoded fluorescent sensors for metabolites to try and identify cellular and molecular mechanisms involved in the energization of neural activity. In this presentation we will explain how these sensors work and give examples of their use in cultured cells and in brain slices. The temporal and spatial resolution afforded by these tools has permitted identifying extracellular K^+ as the main signal linking excitatory synaptic activity to the induction of aerobic glycolysis in astrocytes. Aerobic glycolysis results from concerted stimulations of GLUT1, the glycolytic machinery, and a high throughput lactate channel that surpasses the monocarboxylate transporters. Playing the role of astrocyte-specific K^+ receptors are the Na^+/bicarbonate cotransporter NBCe1 and the Na^+/K^+ ATPase pump α2β2. The strong stimulation of astrocytic glycolysis that results from elevated extracellular K^+ causes a surplus of ATP and acute inhibition of mitochondrial respiration, a phenomenon akin to the Crabtree effect previously described in tumor cells and yeast. In summary, K^+ may be seen as an intercellular signal that shifts oxygen, glucose and lactate from astrocytes to neurons, on demand. Complementary intercellular signaling roles have been proposed for NH_4^+ and NO.

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S09-02

Glia-neuron metabolic interactions beyond lactate

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Neuronal function consumes large amounts of energy. This energy needs to be provided in order to maintain homeostasis. To deal with this disproportionate demand, glial cells support neurons metabolically. Under normal circumstances Drosophila glial cells are very glycolytically active and provide lactate to the neurons. Upon challenges, like starvation or glycolytic impairment, the glial cells turn out to be metabolically very flexible to provide a sufficient amount of highly energetic metabolites to the neurons. They are able to switch to the use of alternative fuels, like lipids. We will discuss mechanisms of adapting metabolic interactions in the nervous system to suboptimal conditions. Further, we give insights into the effect of changes in cerebral metabolism on systemic metabolism and vice versa.

H. Hertenstein and E. McMullen contributed equally.

S09-03

Glial Lactate fuels neuronal activity, a fly view

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Using the powerful genetic toolbox available for *Drosophila*, our lab has identified key monocarboxylate transporters (MCT) that are present in glia cells and in neurons. With these key actors characterized and with the use of genetically encoded sensors for lactate and other metabolites as well as sensors to evaluate neuronal activity, we have characterized the movements of lactate and glucose through the different glia layers in the Drosophila larval brain. Drosophila brain receives metabolites from the hemolymph that bathes the brain but does not enter the brain. Thus, metabolites must cross the blood brain barrier formed by two layers of glia and at least one more layer that surrounds the neuronal body in order to feed neurons. We recorded lactate and glucose fluxes in the *ex vivo Drosophila* brain from larvae and adults during low neuronal activity and during high neuronal activity. Our results support a higher lactate level in glia than in neurons and the need of the lactate flux from glia to neurons to sustain high activity in neurons. Additionally, an essential and active role for the lactate intake by the brain is played by the glia layers of the blood brain barrier, which modulate the expression of MCT transporters during starvation periods. The Drosophila brain show several common features with the lactate transport that is known to occur in mammalian brains, although the glia-neurons interaction is less tight than in mammals and the

availability of oxygen scarcer. Thus, the simplicity of this model system to perform ex vivo experiments, plus its powerful genetic tools represent an opportunity to learn more about the basic principles of the complex metabolic modulation that occurs during neuronal activity, a highly demanding energy process-

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S09-04

Astrocytes store and release lactate on demand: Evidence derived from awake behaving mice.

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We present results using the genetically encoded FRET sensors for lactate and pyruvate *in vivo*. Recombinant adeno-associated virus (AAV) was used with appropriate promoters to express the sensors in astrocytes and neurons. Experiments were carried out in awake, head-fixed mice. Various pharmacological interventions were developed and applied to compare the basal concentration and transients of energy substrates in single cells. We demonstrate that FRET sensors for energy substrates are powerful tools for *in vivo* investigations of the cellular compartmentalization of energy metabolism. We will present evidence for a significant lactate concentration gradient from astrocytes to neurons. This gradient is in support of a vectorial flux of lactate from astrocytes to neurons, as suggested by the astrocyte-neuron lactate shuttle hypothesis. Finally, we demonstrate rapid lactate release from astrocytes upon arousal-mediated cortical activation, followed by lactate production in astrocytes and update in neurons.

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S10 | Where do Glia Come From? Evolution and Diversification of CNS Glia

S10-01

Interlaminar astrocytes evolution in mammals

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Interlaminar astrocytes (ILAs) are a GFAP$^+$ astrocyte subtype in the cerebral cortex that have a soma in layer I and long interlaminar processes that run perpendicular to the pia into deeper cortical layers. We performed a comparative study characterizing ILAs in 46 species encompassing most orders of therian mammals. Wedescribed *rudimentary ILAs* with short processes confined to layer I, and *typical ILAs* with longer and more branched processes exiting layer I and extending into deeper cortical layers. We found that ILAs are present in all mammals, and what makes them special in primates is their highest density and highest morphological complexity.

ILAs have been described in postnatal primate brains, but exactly when they appear during development has not been determined. We studied ILA developmental origin and differentiation of ILAs in the prenatal and postnatal cortex of mouse, rhesus macaque, chimpanzee, and human. We found that ILAs are born prenatally, they proliferate locally after reaching their final destination, and increase in number and morphological complexity throughout development. We compared the expression of specific protein markers in ILAs across development in mouse and macaque and found some similarities in protein expression by mouse rudimentary ILAs and macaque typical ILAs, but noted key differences that may indicate distinct functions across species. These data give precious insights into the phylogenetic relationships among rudimentary and typical ILA, across different orders.

This research will provide new information on ILA astrogenesis and function in the developing cerebral cortex.

S10-02

Glial and microglial functions: connecting the nervous and the immune system in evolution

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The nervous and the immune systems allow us to sense, react and adapt to internal and external stimuli through wired and wireless connections. The intimate relationship between the nervous and the immune systems is highlighted by the presence in the brain of cells of neural origin such as the macroglia (Schwann cells, oligodendrocytes and astrocytes) and cells of immune origin called microglia, which play complementary roles to ensure normal brain structure and function. Macroglia control neuronal development, physiology and survival. Microglia move into the nervous system during development and provide the scavenging function required in physiological and pathological conditions to remove cell debris as well as dead cell bodies.
The Drosophila model has proven its power for understanding the mechanisms underlying the development of glial cells as well as their multiple tasks. Fly glia are of neural origin but also act as the resident scavenger cells of the nervous system during development, upon injury or in pathological conditions. The finding that the fly macrophages acting within (glia) and outside (hemocytes) the nervous system also rely on the same transcription factor for their development raises questions as to the evolutionary conservation of this neuro/immune pathway.
The above findings also suggest that glia are simply non-neuronal cells that take on specific markers and function depending on the neural environment and on the needs. To address this issue, however, we need a clear picture of the transcriptional landscapes present in neural and immune cells. Interestingly, our recent transcriptomic data highlight key molecular features and pathways that are common between fly glia and hemocytes, others that are common between fly glia and neurons and a third molecular signature that specifically characterize fly glia.
Altogether, the data accumulated over the past decades indicate that the evolution of more complex and long-lived organisms has led to a division of labour. This implies the presence of distinct cell types, microglia in the immune system and macroglia in the nervous system, that likely ensure more efficient responses to the internal and external challenges encountered during development and in ontogeny.

S10-03

Evolution of the oligodendrocyte cell type and adaptive myelination phenotype

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All jawed vertebrates (gnathostomes) possess compacted myelin that is structurally and molecularly distinct from invertebrate myelin. Vertebrate CNS myelin is formed by oligodendrocytes, which extend process extensions that wrap target axons to form myelin. It is evident that myelination is a gnathostome novelty, but whether the oligodendrocyte cell type arose prior to or concurrently with the myelinating phenotype is less clear. Cell type identity and cellular phenotypes such as myelination are determined by gene regulatory networks (GRNs) that program the expression of specific combinations of effector genes. The ancestral oligodendrocyte GRN and the

extent it is conserved across gnathostomes is poorly understood. To address this, we have used RNA-Seq analysis to compare the transcriptomes of human, mouse, and zebrafish oligodendrocytes. These comparisons revealed evidence for a conserved gnathostome GRN as well as transcriptional regulators that may represent derived features of mammalian oligodendrocytes. Zebrafish oligodendrocytes expressed 14 of 30 mammalian transcriptional regulators of oligodendrocyte development, and altogether we identified 3,849 protein coding genes with shared expression in human, mouse and zebrafish oligodendrocytes. Functional experiments revealed a conserved role for Myelin Regulatory Factor (Myrf) in gnathostome myelinating oligodendrocytes. Additionally, we identified 16 Myrf orthologous non-coding sequences conserved between cyprinid fishes and are investigating their potential enhancer functions using reporter assays in zebrafish. In this talk I will present these data as well as propose models for the evolutionary origins of the oligodendrocyte cell type and the emergence of the adaptive myelination phenotype.

S10-04

Human-specific gene expression patterns in oligodendrocytes

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Comparative epigenetic and transcriptomic analyses of human and non-human primate brains have proven to be extremely useful in elucidating patterns of molecular innovation unique to the human species. For example, numerous studies have demonstrated extensive modifications of gene expression patterns in human brains compared to chimpanzee brains. While many of these studies using bulk tissue approaches have been biased towards changes in neuronal cell expression patterns, our recent findings using cell-type specific profiling have uncovered an important role for gene expression specifically in human oligodendrocytes that are relevant to cognitive diseases such as schizophrenia. Myelination is a relatively recent evolutionary innovation and white matter volumes can vary in disease-specific ways. Therefore, further dissection of the cellular diversity of oligodendrocytes in human brain evolution and disease is warranted. Using single-cell approaches including snRNA-seq and snATAC-seq across brain tissue from multiple primate species, we have uncovered human-specific patterns of gene expression in subclasses of oligodendrocytes. The maturation of oligodendrocytes is shifted in the human brain permitting greater numbers of cells and changes in gene expression in pre-myelinating oligodendrocyte populations. These changes may indicate altered oligodendrocyte-neuron interaction in the adult human brain with potential consequences in neuronal plasticity and learning. Intersection of these human-specific changes with cognitive disease genetic risk variants has further delineated the importance of these expression changes with respect to disease mechanisms. Together, our data underscore the important contribution of oligodendrocytes to brain evolution and disease.

S11 | Neuron to Microglia communication: neuromodulators and purines get on the front scene

S11-01

Microglia-neuron interactions through somatic purinergic junctions

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Microglia are key regulators of inflammatory processes in the central nervous system and microglial responses are altered in common brain diseases. However, the mechanisms through which microglia contribute to brain homeostasis and brain pathologies are not well understood. We have recently identified a novel form of microglia-neuron interaction, which is present in the majority of neurons in mouse and human brain. Somatic microglia-neuron junctions possess specialized nanoarchitecture optimized for purinergic signaling. We show that the activity of neuronal mitochondria is linked with microglial junction formation, which is induced rapidly in response to neuronal activation and blocked by inhibition of P2Y12 receptors. Brain injury-induced changes at somatic junctions trigger P2Y12-dependent microglial neuroprotection, regulating neuronal calcium load and functional connectivity. Our results suggest that motile microglial processes exert fine-tuned control of neuronal actions in the healthy and the injured brain. Understanding the mechanisms of microglia-neuron interactions is likely to help the identification of novel therapeutic targets in common neurological disorders.

S11-02

Microglial dynamics impact synaptic surveillance and plasticity

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The ability to tune synapses, and thus alter neural networks, is critical to both the normal development of brain circuitry and brain function throughout life, underlying processes such as learning and memory. Microglia are immune cells that infiltrate the brain early in development before the formation of the blood brain barrier. They have critical roles during brain injury, infection or disease. However, new data has thrust these non-neural cells into the spotlight as regulators of synapses. In the absence of pathology, microglia display dynamic interactions with

synapses, and contribute to experience-dependent plasticity in the visual cortex in vivo. Manipulations of visual experience elicit a remarkably rapid behavioral response in microglia which is distinct from their inflammatory response, and includes an increase in phagocytosis that corresponds to the early phase of plasticity when synapses are lost in this model and when microglia increase their synaptic interactions. Our recent work has focused on identifying the molecular mechanisms through which microglia respond to changes in neuronal activity and communicate with synapses. Purinergic signaling through the microglial P2Y12 receptor and adrenergic signaling through the microglial beta2 adrenergic receptor appear to have opposite effects on microglial physiology and dynamics and both impact microglial interaction with synapses and experience-dependent plasticity. Together, our findings suggest that microglia play an important role in synaptic plasticity, and use a subset of their pathological molecular repertoire to implement plastic changes in the non-pathological brain.

S11-03

Serotonergic control of microglia during development controls innate behaviors and response to systemic inflammation in adulthood

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Serotonin (5-HT), besides its role of neuromodulator in adulthood, is a neurodevelopmental factor involved in the postnatal formation of somatosensory and emotional circuits. Much effort has been dedicated to understand how 5-HT plays this role by acting on neurons, but we and others have shown that microglial cells also express serotonin receptors, mainly the 5-HT_{2B} subtype[1,2], with an increasing expression from birth to P15[3]. In addition, a focal application of 5-HT triggers an oriented growth of their processes, and microglia lacking the 5-HT2B receptor have an abnormal inflammatory response in vitro[1], suggesting a regulatory role of 5-HT on microglia. To investigate the control of microglia by 5-HT during postnatal development, we compared the impact of deleting *Htr2b* gene in microglia only, since birth or adulthood. We observed that an early deletion of *Htr2b* impacts on microglia development, synapse density and axonal refinement during adolescence. Absence of this early serotonin-microglia interaction has also detrimental consequences on some innate behaviors and on the response to a systemic inflammation in adulthood[3]. In contrast, invalidation of *Htr2b* in adult microglia has no effect on these behaviors, which confirms the role of microglia and 5-HT in neurodevelopment and suggests that a neonatal instruction of microglia by 5-HT is required to allow their normal maturation and functions from birth to adulthood.

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S11-04

Neuronal regulation of microglial TNF release controls synaptic strength and circuit function

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Microglia are responsive to a number of extrinsic and intrinsic signals that modulate their activation state and regulate the production of inflammatory cytokines including tumor necrosis factor alpha (TNF). The release of TNF, in turn, regulates the excitability of neighboring neurons by inversely altering the strength of both glutamatergic and GABAergic synapses. Our recent work has revealed that microglia respond to a number of neuromodulators by shifting the production of TNF, and this contributes to a number of induced behaviours. In particular, microglia TNF is an important regulator of addiction-induced behaviours as well as stress-induced anxiety. In the context of addiction, we have shown that the increase in dopamine extracellular concentration upon cocaine injection directly stimulates microglia and induces their production of TNF, and that this process limits the development of drug sensitization. On the other hand, microglia TNF appears to drive the development of anxiety following stress. This works demonstrates that microglia could thus be key intermediates for a variety of behavioural changes and potential therapeutical targets in some of the effects of neuromodulators.

Acknowledgement

This work was funded by the Canadian Institute of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC).

S12 | Metabolic aspects in myelinating glia: implications for health and disease

S12-01

The role of autophagy in the development and maintenance of myelin in oligodendrocytes

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Autophagy comprises a major lysosome-dependent degradation mechanism which engulfs, removes, and recycles unwanted cytoplasmic material, including damaged organelles and toxic protein aggregates. Although a few studies implicate autophagy in CNS demyelinating pathologies, its role, particularly in oligodendrocytes and CNS myelin, remains poorly studied.

We will present data on the significance of macroautophagy in the nervous system, focusing on myelinating glia of the CNS. We investigate the role that this catabolic process plays in myelin homeostasis with the ultimate goal to reveal novel therapeutic targets for the treatment of central demyelinating pathologies, including MS.

To this end, we have used both *in vitro* and *in vivo* approaches. *In vitro*, pharmacological and genetic inhibition of autophagy have revealed severe defects in myelin sheet formation, delayed maturation and altered cellular distribution of major myelin protein constituents. In parallel, we are currently examining the role of autophagy *in vivo*, utilizing a new conditional mutant mouse line, in which a core gene of autophagic machinery (*atg5*) is specifically ablated in the myelinating glial cells after tamoxifen administration (*plp-Cre^{ERT2}; atg5 $^{fl/fl}$*). Biochemical and ultrastructural analysis of this mouse line has revealed differences in myelin protein levels as well as morphological alterations in cKO animals compared to age-matched controls.

Our data support the novel principle that the progression of myelination in the CNS requires the involvement of a fully functional autophagic machinery, thus it is expected that manipulation aiding this process could provide an innovative and effective therapeutic approach for myelin disruption.

Acknowledgement

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S12-02

Schwann cells autophagy: fast and immediate clearance of aggresomes for counteracting peripheral neurodegeneration.

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Schwann cells (SCs) are essential for reparative process after peripheral nerve injury to control Wallerian degeneration (WD), which involves the progressive demyelination of peripheral nerves. Schwann cells and resident immune cells represent the first players in mediating early inflammatory response after nerve damage.
When WD begins, SCs degrade and remove degenerated axons and myelin throughout SCs autophagy, a process recently defined as myelinophagy. In fact, thanks to their peculiar adaptive and flexible behaviour, after nerve lesion, SCs modulate their phenotype showing and acquiring increased capacity to clear aggregosomes by an nhancement of autophagy. Autophagy is a cellular self-preservation mechanism by which pathogens, dysfunctional cells as well as parts of them are phagocytosed, degraded and recycled for cellular repair or utilized as energy during starvation.
In the last years different studies have focused on the role of autophagy in neuronal injury. It has been demonstrated that autophagy process in SCs plays a key role in myelin debris clearance during the first days after nerve injury, demonstrating that defects in SCs autophagy performance, such as after pharmacological manipulation, genetic and metabolic alterations or ageing, leads to a lack in neuropathy and functional recovery, in inflammation increasing, exacerbation of allodynia and to neuropathic pain chronification.
These evidence suggested that autophagy activation and/or stimulation could promote myelinogenesis and selective anti-inflammatory mechanisms. Since autophagy machinery can be triggered by a plethora of stimuli, both the specific autophagy inducer rapamycin and caloric restriction to mimic starvation, have been utilized as strategy to prompt myelinophagy in murine models of neuropathy, demonstrating that this can represent a therapeutic intervention in peripheral neurodegeneration.

S12-04

Single-cell Sequencing Reveals Brain/Spinal Cord Oligodendrocyte Precursor Heterogeneity and Requirement for mTOR in Cholesterol Biosynthesis and Myelin Maintenance

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Brain and spinal cord oligodendroglia have distinct functional characteristics, and cell autonomous loss of individual genes can result in different regional phenotypes. However, sequencing studies to date have not revealed distinctions between brain and spinal cord oligodendroglia. Using single-cell analysis of oligodendroglia during developmental myelination, we have defined cellular mechanisms which establish distinctions between brain and spinal cord oligodendroglia. One of the most striking distinctions is that oligodendrocyte precursor populations in spinal cord exhibit higher levels of cell-autonomous cholesterol biosynthesis than the equivalent stage precursors in the brain. Conversely, brain oligodendroglia have a higher capacity for extracellular cholesterol uptake. Our data also reveal that the mechanistic target of rapamycin (mTOR) promotes cholesterol biosynthesis in oligodendroglia. The differential dependence on endogenous cholesterol biosynthesis in spinal cord and brain oligodendroglia suggests an explanation for our observation that oligodendrocyte-specific loss of mTOR has a greater impact on developmental myelination in the spinal cord compared to the brain. However, loss of mTOR in brain oligodendroglia ultimately results in oligodendrocyte death, spontaneous demyelination, and impaired axonal function, demonstrating that mTOR is required for myelin maintenance in the adult brain.

S13 | Advances in cellular reprogramming: where biology meets physics

S13-01

Mathematical models of cellular identity converge with models of neural networks and provide framework for cellular identity

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Cell types are defined by relatively stable gene expression patterns. Since gene expression is controlled by transcription factors, which are proteins or protein complexes, and since protein concentrations are controlled by gene expression, we are studying a system with prominent feedback loops. These loops are essential for the system to be able to have multiple locally stable states; without feedback loops there would be at most one stable state, and hence no multiple cell types. The proteins and their complexes act as middlemen for the effective interaction between gene expression variables, but this mediation is not one-to-one. Proteins and their complexes are subject to biochemical reactions in the proteome, and the concentrations of transcription factors are therefore not simply proportional to the expressions of the genes from which they are produced. Any reasonable mathematical description must describe both of the above processes in order to capture the feedback loops, and hence the multiplicity of locally stable states. However, one can benefit from the fact that proteomic processes are much faster than the transcription processes. This allows one, given certain simplifying assumptions, to integrate out the fast variables, and derive an effective mathematical description that involves only the gene expression levels, valid on the larger timescales where cell identity can change. If one carries out this reduction one finds that the resulting mathematical equations bear a remarkable resemblance to the types of equations that have been used to describe signalling in recurrent neural networks. The gene expression levels are the equivalent of the neuronal firing rates, and the effective interactions between genes are the equivalent of the synaptic junctions between neurons. This link between mathematical models of gene regulation networks and recurrent neural networks is very useful. In recurrent neural networks, information is stored in the form of attractors (i.e. locally stable firing states of the neurons), and the precise nature and strengths of these attractors (the information processing `program' of the network) is controlled by the synapses. Over several decades we have learned a lot about how one should manipulate the synapses in order to achieve any predefined macroscopic dynamical behaviour in the system, or to create specific stable states. In other words, we know how to `reprogram' the network by intelligent manipulation of specific local control variables. The mathematical similarity with gene regulation networks suggests that we may be able to use the same formalism and rules to `reprogram' the transcriptome, where attractors play the role of cell types. This presentation will explain the basic ideas.

S13-02

An integrated multi-omics-analysis provides mechanistic insight into the process of iPSC-to-neuron reprogramming.

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Direct cell reprogramming, such as the transition of iPSCs into hiNeurons following expression of Neurogenin 2 (NGN2) challenges traditional concepts of cellular identity. We have previously demonstrated that gene targeting the components of a Tet-On system into two separate safe harbour sites overcomes gene silencing, results in optimised transgene expression in hiPSCs (OptiOx), and yields highly homogenous cultures of pure (>99.999%) neurons within less than four days. The mechanisms that mediate this remarkable cellular metamorphosis are poorly understood.

To study the transcriptional and epigenomic that govern this process we conducted a detailed time course experiment covering the reprogramming from iPSCs into hiNeurons (day0-4) and their subsequent maturation (day4-21), including bulk RNA and ATAC sequencing, scRNA-Seq and NGN2-Chip-Seq.

This revealed rapid transcriptional and epigenetic changes induced by NGN2 within 6 hours of reprogramming. Cells synchronously transition through distinct stages with little heterogeneity until attaining a distinct neuronal phenotype. During reprogramming, cells transition through transcriptional stages resembling developmental neurogenesis. Non-neuronal gene networks associated with pluripotency were rapidly down-regulated and a NSC-like stage was established by Day 1 post-induction. Cells exited cell cycle by Day 3 or Day 4 and established neuronal specification. This was followed by neuronal maturation.

ScRNAseq analysis showed that in addition to glutamatergic neurons, NGN2 encodes cholinergic neurons with a visceral motor phenotype and neurons with a hybrid profile of cholinergic and glutamatergic.

Reconstruction of the transcription factor network that governs cell reprogramming from transcriptional and epigenomic datasets was supplemented with CHiP-Seq data acquired on day 2 after to identify direct and indirect effectors of NGN2. Ongoing CRISPRa and ko studies seek to validate the TF networks driving reprogramming.

In conclusion, the transition of iPSCs into functional neurons following NGN2 expression serves as a valuable model system to study concepts of cell identity. Our data indicates that 1. NGN2 encodes an albeit limited range of neuronal identities; 2. during reprogramming cells transition through stages that resemble development; 3.this process is highly homogeneous and involves 4. rapid and wide-ranging transcriptomic as well as epigenetic changes; 5. neuron-specific transcriptional networks are established within 3-4 days, whilst 6. closure of chromatin mainly occurs after neuronal specification of cells.

Acknowledgement

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S13-03

Programming human pluripotent stem cells into microglia – a new model to study CNS disease

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Microglia, the resident immune cells of the central nervous system (CNS), are derived from yolk sac macrophages that arise during the first wave of primitive haematopoiesis and populate the developing CNS during early embryonic development. Once established, the microglia population is self-maintained throughout life by local proliferation. The generation of microglia-like cells from human pluripotent stem cells (hPSCs) is mostly performed using classical differentiation protocols, which follow the events of embryonic development. They provide a valuable tool for the scalable production of human microglia-like cells for drug discovery and disease-modelling. However, published protocols are characterised by long culture durations (up to 75 days) and the need for enrichment steps of intermediate progenitor populations, thus hampering their widespread application.

We present a forward programming approach for the generation of microglia-like cells from hPSCs based on transient overexpression of master reprogramming factors in hPSCs in conjunction with specific extracellular cues. This robust, scalable protocol yields pure populations of microglia-like cells in less than three weeks and does not require enrichment of intermediate cell types. The microglia-like cells meet microglia characteristics on a morphological, genetic and functional level.

These microglia-like cells represent an excellent tool for both reductionist studies in monocultures and complex co-culture systems including 3D brain organoids for the study of cellular interactions in healthy or diseased environments.

S13-04

Next generation cell therapies for the brain

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Pelizaeus Merzbacher disease (PMD) is a hypomyelinating leukodystrophy caused by mutations in the proteolipid protein 1 (PLP1) gene, encoding an abundant myelin protein. Failure in oligodendrocyte precursor cell (OPC) maturation and myelination causes profound axonal dysfunction, motor deficits, developmental delay and lethality before adulthood1. Experimental models suggest mutant PLP1 protein causes OPC cell death by triggering the

unfolded protein response (UPR) and endoplasmic reticulum (ER) stress pathways. However, the variable nature of PLP1 genotype-phenotype association in PMD suggests the possibility of other patient-specific pathobiological mechanisms. Here we used patient-derived induced pluripotent stem cells (iPSCs) and genetically corrected, isogenic cells to assess the impact of PLP1 mutations from two severely affected PMD patients. Genetically corrected iPSC-derived OPCs showed functional correction in vivo as seen by restorative myelin formation after transplantation into myelin-deficient mice. These data provide proof of concept that a combination of reprogramming, gene engineering, and differentiation is a therapeutic path for myelin disorders. We also observed that PLP1-mutant, iPSC-derived iOPCs were formed normally but underwent apoptosis upon further differentiation. Unexpectedly, UPR and ER stress pathways were not activated as the mutant protein was transported to the cell membrane. Instead, we found that mutant cells were critically sensitive to iron which caused secondary lipid oxidative stress. Gene-corrected OPCs were largely insensitive to increased iron. Pharmacological iron chelation rescued mutant cells in vitro and upon transplantation into hypomyelinating mice. OPCs from PLP-mutant jimpymice also showed enhanced survival and differentiation upon iron chelation.

In a second approach, we have investigated the use of bone marrrow transplantation to replace endogenous microglia with circulation-derived myeloid cells (CDMCs). We found that liberating microglial niche factors by microglia depletion is critical for robust differentiation and incorporation of CDMCs. Consequently, a combination of bone marrow transplantation conditioning and pharmacological depletion of microglia with a CSF1 receptor inhibitor resulted in near complete replacement. We are currently seeking to understand the cellular mechanisms of CDMC recruitment and to evaluate proof of principle therapeutic potential of this impressive brain reconstitution.
In summary, our findings reveal two prominent glial cell types with intriguing potential to modify disease pathogenesis or restoration of function. The combination of cellular reprogramming and gene editing are a powerful tools to develop next generation cell therapies for the brain.

S14 | Perisynaptic astroglial processes: activity-dependent regulation and function

S14-01

Investigating astrocytic Ca^{2+} signals with STED and lattice light sheet microscopy

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Astrocytic Ca^{2+} signals can be fast and local, supporting the idea that astrocytes have the ability to regulate single synapses. However, the anatomical basis of such specific signaling remains unclear, owing to difficulties in resolving the structure of tripartite synapses with regular light microscopy.

Using 3D-STED microscopy in living organotypic brain slices, we observed that the spongiform domain of astrocytes was composed of round enlarged nodes and thin shafts, which often formed ring-like structures. FRAP experiments and Ca^{2+} imaging showed that nodes were biochemical compartments and Ca^{2+} microdomains. Mapping astrocytic Ca^{2+} signals onto STED images of tripartite synapses showed that node Ca^{2+} signals were associated with individual synapses, identifying nodes as the likely functional astrocytic component of tripartite synapses.

To further characterize the properties of nodes, we recently established ultra-fast Ca^{2+} imaging in 2D and 3D using lattice light-sheet microscopy, which reconciles high temporal and spatial resolution with low phototoxicity. We show that node Ca^{2+} signals can be as transient as < 100 ms and can be highly restricted in space. Finally, we show that glutamate uncaging elicits Ca^{2+} responses in nodes, indicating that nodes are equipped with the signaling machinery to respond to synaptic activity.

Our combined super-resolution and light-sheet imaging approaches open up the possibility to dissect the bidirectional crosstalk between the neuronal and glial elements of tripartite synapses, and evaluate its impact on neural circuit function with unprecedented spatial and temporal resolution and sensitivity.

Acknowledgement

The study was supported by research grants from the Agence Nationale de la Recherche (ANR-12-BSV4-0014 and ANR-17-CE16-0002) and Fondation pour la Recherche Médicale (DEQ20160334901) and LabEx BRAIN. M.A. was supported by postdoctoral fellowships from RIKEN and JSPS (Japan).

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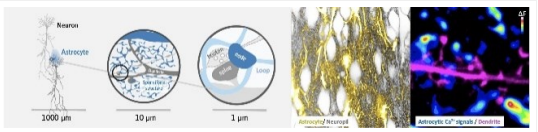

Summary of the study

S14-02

Activity-dependent regulation of the molecular repertoire in perisynaptic astroglial processes

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Astrocytes are active protagonists in brain information processing, from synaptic plasticity to neuronal network oscillations and memory function. Probing astrocyte function is usually performed at the level of the entire cell. Yet a single astrocyte can contact thousands of synapses, and signal integration at the whole-cell level is only partially informative about fine neuron-astrocyte interactions. Therefore, deciphering how astrocytes integrate and respond to neuronal activity requires focusing on their smallest compartments, the perisynaptic astrocytic processes (PAP). PAP are nanoscopic motile elements that dynamically enwrap synapses and can sense their activity via local expression of a wide variety of neurotransmitter receptors and ionic channels. Beyond structurally interacting with synapses, they are also packed with neurotransmitter transporters, promoting synapse independence and are a source of molecular signals regulating synaptic transmission. Yet, the modalities of neuroglial interactions at the nanoscopic level of PAP-synapse interface are still poorly understood. Here, I will present data showing the occurrence of local translation in PAP from the hippocampus and identifying PAP molecular repertoire, as well as its dynamic regulation by activity and behaviour. These molecular data point to a role for astroglial local translation in learning and memory.

S14-03

Activity-driven plasticity of perisynaptic astroglial environment

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Point-to-point transmission in central excitatory circuits depends on rapid uptake of glutamate by high-affinity transporters expressed in perisynaptic astroglial processes (PAPs). Remodelling of synapses underlies formation of memory trace in the brain, but whether and how this remodelling affects astroglial synaptic environment, hence extrasynaptic and possibly inter-synaptic actions of glutamate, remains a subject of debates. Resolving this long-

standing issue has been a challenge because the nanoscopic dimensions of PAPs are beyond the diffraction limit of traditional optical microscopy. We used several imaging methods that are independent of light diffraction to find that a classical mechanism of synaptic memory, long-term potentiation (LTP), triggers the shrinkage of PAPs prompting their withdrawal away from potentiated glutamatergic synapses. Patch-clamp electrophysiology combined with optical glutamate sensors and super-resolution 3D dSTORM imaging in acute hippocampal slices reveal that LTP induction thus initiate spatial retreat of perisynaptic astroglial glutamate transporters, boosting glutamate spillover and NMDA receptor-mediated inter-synaptic cross-talk. The underlying cellular cascades involve NKCC1 transporters and the actin-controlling protein cofilin. The LTP-triggered PAP withdrawal can also be triggered by the whisker-stimulation induced LTP in the barrel cortex in vivo. In these in vivo settings, we also detect increased glutamate escape following LTP induction, by using a novel multiplexed imaging method that enables tracking synaptic efficacy changes and glutamate spillover at individual synapses in awake animals. These findings uncover a mechanism by which memory trace induction alters signal integration rules in the astroglial environment of excitatory synaptic circuits.

Acknowledgement

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S14-04

Proteomic and functional analyses of perisynaptic astroglial process dynamics

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Astrocytes have been found structurally associated with neuronal synapses and to actively control synaptic function via exchange of regulatory signals. Whereas much of the molecular processes in the neuronal pre- and postsynaptic elements have been identified and insight into their role in functioning of the synapse is increasing, little is known about the astrocytic machinery in perisynaptic astroglial processes (PAPs) and their functional interaction with synapses. Here, we used mass spectrometry-based proteomics to identify a set of putative PAP proteins. Next, fear conditioning (FC), a well-established paradigm for hippocampus-dependent contextual memory, was used to investigate PAP proteins regulated during synapse consolidation. Interestingly, FC-induced regulation of PAP proteins was accompanied by morphological changes in PAPs, as identified with electron microscopy (EM). Manipulation of PAPs was done by viral Crispr-Cas9 mediated targeting of Ezrin in astrocytes in the adult mouse hippocampus. We conclude that memory consolidation is associated with dynamic changes in PAP morphology and PAP protein levels. Ongoing experiments are focused on the functional consequences of manipulating specific PAP proteins to dissect their implication in synaptic transmission and memory.

S15 | Glial modulation of circuit assembly and behavior: insights from different model organisms

S15-01

C. elegans glia drive brain assembly through specialized morphogenesis and crosstalk with neurons

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Early assembly of functional nervous systems relies on intricate interactions of neurons and glia, both essential sculptors of circuits. Despite extensive studies of neurodevelopment, the events initiating circuit assembly and the underlying glia-neuron crosstalk are challenging to examine. The properties, behavior and molecular mechanisms of cells that form early paths to pioneer circuit assembly remain elusive. Glia appose to axons early but our understanding of their roles in circuit initiation remains cursory. Which events of glia-neuron communication and morphogenesis pioneer brain circuits? Which genetic pathways and molecular mechanisms drive these events *in vivo*?

Research in model organisms suggests that core elements of glial biology are conserved and allows for advanced gene-function analysis to dissect glia roles and interactions. We study glia-neuron crosstalk and development using primarily *C. elegans*, a powerful model allowing live embryonic imaging, advanced genetics and molecular tools. The *C. elegans* nervous system harbors a defined number of individually-identified glia with important similarities to vertebrate glia and dispensable for neuronal viability, a key advantage to study their early developmental roles. Exploiting these advantages, we examine how glia-neuron crosstalk and development shape brain circuit assembly.

We have uncovered that specific glia drive the assembly initiation of the *C. elegans* brain-like neuropil. These CEPsh glia grow membrane processes to demarcate the early brain circuit paths and guide pioneer and follower axons, through distinct conserved pathways. We describe how embryonic CEPsh glia resemble vertebrate radial glia and later transform into tufted astrocyte-like glia with elaborate processes, shown to ensheath and regulate synapses. We investigate how CEPsh glia interact *in vivo* with neurons and develop their specialized morphology, focusing on the underlying molecular mechanisms and tissue interactions. We combine powerful approaches of advanced genetics, live imaging,and cell manipulations. Through unbiased screens and candidate approaches, we identify an array of genes that control early interactions and morphogenesis of pioneer glia and neurons. Many of these conserved genes have homologs implicated in pathologies, ranging from birth defects to autism, epilepsy, Alzheimer's disease and cancer. We will investigate the roles of these genes in early brain development with high spatiotemporal resolution *in vivo*, a previously unmet challenge. Alongside mouse studies, our work suggests conserved glia-neuron interactions in early circuit architecture. Studying the development of pioneer glia and neurons may illuminate conserved aspects of glia biology and circuit assembly.

S15-02

Glia-MB acetylcholine-mediated synaptic connections underlie *Drosophila* long term memory

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Glia has been viewed as passive bystanders that provide support to neuronal network. In the past decades, tremendous progress has been made on how glia actively regulates virtually all aspects of neuronal function. Using *Drosophila* as a model, we uncovered a synaptic-like interaction between astrocytes and the α/β Kenyon cells in the olfactory-associative learning center Mushroom Body (MB). Interestingly, this interaction requires specific α1 and α5 subunits of glial nicotinic acetylcholine (ACh) receptors (nAChR). Exogenous ACh application activates astrocyte calcium responses, suggesting that the transmission from MB neurons to astrocytes is mediated by ACh. Furthermore, blocking nAChR expression in glia causes defects in appetitive long-term memory (LTM) formation. Taken together, these findings implicate a role for astrocyte glia regulating potential MB synaptic activity during *Drosophila* long term memory.

S15-04

Astroglial control of *C. elegans* behavior

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Astroglia, glial cells that tightly associate with neurons, are implicated in the control of many behaviors, and their dysfunction accompanies pathological conditions manifesting behavioral abnormalities. Yet, neural circuit mechanisms by which astroglia affect behavior are largely unknown. The nematode *C. elegans,* with its simple nervous system and well-mapped connectome, is an attractive setting for deciphering such mechanisms. *C. elegans* CEPsh glia, which associate with neuronal processes and synapses in the animal's brain, resemble mammalian astroglia in morphology, molecular biology, and function. We recently uncovered a key role for CEPsh glia in controlling repetitive behavior in *C. elegans*. Loss of the glutamate transporter GLT-1, enriched in CEPsh glia and in mouse astrocytes, causes glutamate spillover, leading to ectopic presynaptic activation of the neuronal metabotropic glutamate receptor MGL-2/mGluR5. This, in turn, induces postsynaptic neuron oscillations, generating abnormal repetitive backing behavior. Importantly, murine GLT1 and mGluR5 are implicated in pathological motor repetition, suggesting that conserved mechanisms control repetitive behavior generation from *C. elegans* to mammals. Our studies establish *C. elegans* CEPsh glia as an exciting model to study fundamental molecular and cellular roles of astrocytic glia in neural circuits.

S16 | Microglia; from development to ageing

S16-01

The role of glia cells in Alzheimer's Disease

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Although complex inflammatory-like alterations are observed around the amyloid plaques of Alzheimer's disease little is known about the molecular changes and cellular interactions that characterize this response. We investigated in an AD mouse model the transcriptional changes occurring in tissue domains in a 100-mmdiameter around amyloid plaques using spatial transcriptomics. We demonstrated early alterations in a gene co-expression network enriched for myelin and oligodendrocyte genes (OLIGs), whereas a multicellular gene co-expression network of plaque-induced genes (PIGs) involving the complement system, oxidative stress, lysosomes, and inflammation is prominent in the later phase of the disease. We confirm the majority of the observed alterations at the cellular level using in situ sequencing on mouse and human brain sections. Genome-wide spatial transcriptomics analysis provides an unprecedented approach to untangle the dysregulated cellular network in the vicinity of pathogenic hallmarks of AD and other brain diseases.

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S16-02

Human microglia in the healthy and diseased CNS

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Microglia are the resident macrophages of the central nervous system (CNS), which are important for CNS development and tissue homeostasis. During development, colonization of the developing CNS by microglia precedes neurogenesis, and they regulate the expansion and differentiation of neural precursors. Microglia are implicated in circuitry formation by synaptic pruning, and in the adult CNS their ramified and highly motile processes continuously surveil their immediate surroundings and quickly respond to homeostatic disturbances.

With the emergence of tools to deep phenotype individual cells, insights in the role of microglia in CNS development, homeostasis, and disease has been obtained. Microglia properties and heterogeneity in the human brain, during fetal development and in adulthood, and how they are affected in CNS disease will be discussed.

S16-03

A human(ized) system to study microglial biology *ex vivo*

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Converging evidence suggests an involvement of microglia and inflammation in the onset of many neurodegenerative diseases. Most of our knowledge on microglial biology derives from rodents or primary cell cultures and remain to be translated to human, highlighting the need for a human(ized) system.
Inaccessibility of living human brain tissue has limited the number of studies investigating neurons and glia and their respective roles. Analyses of postmortem tissue can by default only provide a snapshot of glial phenotypes, thereby neglecting early cellular responses to pathological alterations.
We developed an *ex vivo* mouse brain slice culture system in combination with human induced pluripotent stem cells (iPSC)-derived microglia as a novel cellularly complex tool to dissect the dynamics and molecular mechanisms of genetic risk factors of neurodegeneration. We found that iPSC-derived microglial precursors integrate and differentiate well in the brain slice cultures, with morphology, network characteristics and functional responses reminiscent of human microglia.
This system is a novel cellularly complex tool allowing to dissect the dynamics and molecular mechanisms of genetic risk factors of neurodegeneration in unprecedented detail.

S16-04

Microglia shape the development of neuronal network

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Microglia cells are present around neural cells from early development, throughout life. The association of microglia expressed genes with developmental, psychiatric and neurodegenerative conditions suggest various important

roles in brain physiology. Their function ranges from phagocytosis, immunological functions, and neuronal support, to synapse pruning. As the function of microglia is context dependent, it is important to understand how extracellular stimuli can regulate this.

The absence of microglia in early embryonic neocortex results in abnormal development. In order to study human microglia function in early development, we took advantage of directed differentiation of pluripotent stem cells to yolk sac derived microglia and various neural cell types to create an *in vitro* neural tissue model. Using the *in vitro* cortical developmental model, we can correlate transcriptomic and functional changes of neural and microglia cells. We found that cortical deep layer neurons induce microglia maturation of yolk sac macrophages, that in turn stimulate neuronal maturation and synapse formation, leading to synchronized network activity. Our model captures a novel synaptogenic microglia cell state and highlights a time window during development during which microglia can enhance neuronal circuit formation.

S17 | Mechanics of glial cells in development and disease

S17-01

Mechanosensing Controls Myelin Sheath Elongation in the CNS

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Recent work indicates that variation in myelin sheath lengths in the CNS may be a mechanism to coordinate the timing of neuronal signaling to ultimately impact behavior. Myelin sheath lengths vary substantially in the CNS, and together, this has led to the recent hypothesis that myelin sheath lengths are important for adjusting signaling speeds rather than maximizing them. Thus far, however, there is little understanding of how myelin lengths are determined. In vivo, myelin sheath size often scales with the caliber of the axons that are being ensheathed, but the mechanism for this increase in myelin sheath size relative to the axon size is unknown. Our previous work using a 3D neuron-free microfiber culture system demonstrated that myelin sheath lengths can be oligodendrocyte-driven. We identified two contributing factors to oligodendrocyte self-regulation of sheath length: cellular origin and diameter sensing. Our recent work has focused on how oligodendrocytes respond to diameter to control different myelin sheath lengths. We find that diameter sensing and the regulation of sheath length is precisely localized; each individual nascent myelin sheath responds to underlying fiber diameters. Using a candidate-based screen, we uncovered a new role for a mechanosensitive ion channel in tuning the length of myelin sheaths to fiber diameter. In vivo, this ion channel impacts the elongation of myelin sheaths on large caliber axons. We propose that this mechanosensitive ion channel provides a mechanism for the ability of oligodendrocytes to scale sheath length with substrate caliber, to produce myelin segments with vastly different lengths.

S17-02

Brain tissue mechanics and mechanobiology of glial cells

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The brain is one of the softest tissues in the body. When subject to different loading conditions, it responds in the time-dependent manner and when under *compression*, *tension* or *shear* it behaves as non-linear, viscoelastic body (3). Most importantly, brain tissue compression-stiffens, what proves that cells within brain tissue are exposed to physical forces and stiffnesses higher than we could expect (1). Therefore, it is worth studying how glial cells adopt to an increased stiffness of their surrounding and whether this implies changes in their fate and function (2). In parallel, brain is also highly viscous and glial cells response to viscosity of their surrounding is also of high demand.

Recent development of soft viscoelastic materials where the elastic and viscous moduli can be independently tuned has opened up the possibility to characterize the impact of both elasticity and viscous dissipation on malignant brain cells (5). The potential of mechanical stimuli to directly influence cell function are particularly interesting in the context of brain tumor growth and are essential for understanding how cells and tissues develop under normal conditions and how they change when exposed to altered mechanical loads. Not only increased stiffness but also internal stresses arise under compressive force, hence increased solid tissue stress inside the tumor mass cause preferential deformation of the non-tumorous margin what results in neuroinflammation and reduced vessels perfusion (4). This implies, that the mechanical characterization of the brain and further investigation of the mechanobiology of glial cells under active mechanical forces have both diagnostic and therapeutic relevance.

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S17-03

Machine learning in direct glia-to-neuron reprogramming to a neuronal fate: Learning from cell mechanics for fate prediction

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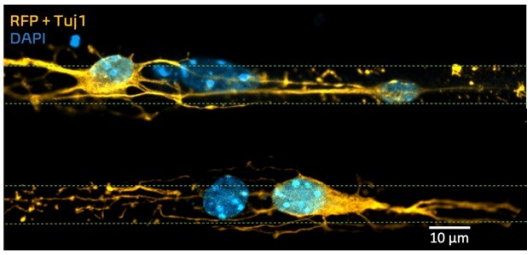
Direct glial reprogramming into induced neurons is a promising therapeutic strategy to repair dysfunctional brain circuits. During reprogramming of cell identity, remodelling of gene expression must be matched by a complete turn-over of cellular properties. This radical turn-over is strikingly reflected by alterations in shape, size, and chromatin distribution of the nucleus along with cell fate conversion. Here, I will provide evidence from *in vitro* studies highlighting interactions of cells undergoing reprogramming with their microenvironment and discuss cell mechanical aspects underlying successful transformation. I will illustrate nuclear structural changes during the reprogramming of glial cells into neurons and discuss how these can be a physical roadblock that only a few cells overcome. Moreover, I will describe how morphological changes of the nucleus and chromatin distribution can be captured using high-content image analysis. Using these data, we employ multiparametric machine learning methods to predict distinct cell fate decisions successfully (e.g. failed conversion, successful conversion, cell death) along the reprogramming trajectory with high fidelity. Our data, together with a recent surge in studies aimed at understanding the role of biomechanics in regulating cell fate decisions call for rethinking strategies for improved direct lineage reprogramming.



Glia-to-neuron reprogramming on 10um thick line patterns

S17-04

How do oligodendrocytes add new membrane to build the myelin sheath?

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Myelin accelerates conduction velocity by insulating axons in spiraling layers of glial plasma membrane, which are produced by oligodendrocytes in the central nervous system. Generating massive volumes of new myelin membrane during development require high rates of membrane expansion in oligodendrocytes. Components that mediate membrane fusion for exocytosis have been implicated in the transport of myelin membrane proteins. However, the required components for myelin membrane expansion remain unclear. Here, we use transgenic mice to inactivate vesicle-associated membrane proteins (VAMP) 2 and 3 specifically in differentiating oligodendrocytes during development. Disrupting exocytosis in oligodendrocytes resulted in hypomyelination and shortened myelin sheaths, ultimately preventing the formation of mature Nodes of Ranvier during development. Using primary oligodendrocyte culture, we find that inactivating exocytosis in differentiationg oligodendrocytes inhibits membrane expansion without inhibiting differentiation, and prevents myelin sheath elongation in myelinating co-cultures with neurons. Our study indicates a required role of exocytosis for myelin sheath elongation and identifies VAMP2 and 3 as components of the membrane fusion machinery for myelin membrane expansion. Whether VAMP2 and 3 in oligodendrocytes coordinate membrane fusion with neuronal activity is an active area of investigation.

Acknowledgement

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S18 | Targeting glial cell activation for treatment of neurodegenerative disease

S18-01

Neuroimmune cell atlas for the human brain reveals gene networks activated in neurological disorders

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I will describe at atlas of neuroimmune cell diversity across the human lifespan from gestation to old age, leveraging >5 million human brain single-cell transcriptomes and epigenomes from the United States' Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative Cell Census Network. Microglial subsets up-regulate cytokines, phagocytic genes, and other specialized gene networks in pleiotropic contexts throughout life. Distinct microglial gene networks are activated in neurodevelopmental vs. neurodegenerative disorders.

Acknowledgement

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S18-02

Targeting a signal of glial activation: clinical evaluation of Semaphorin 4D blocking antibody pepinemab

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Drivers of glial cell activation may represent important targets to preserve normal homeostatic maintenance and modify progression of neurodegenerative pathology. Semaphorin 4D (SEMA4D) is upregulated in neurons in response to stress, and SEMA4D binding to its cognate plexin B receptors regulates microglial activation, survival and differentiation of glial precursor cells, disruption of the neurovascular unit, and astrogliosis with concomitant loss of some normal astrocyte functions. SEMA4D induced downregulation of astrocytic glutamate and glucose

transporters, EAAT-2 and Glut-1, while antibody blockade inhibited these effects and reversed deficient glucose uptake in vitro. In vivo antibody treatment normalized neuroinflammatory pathology and ameliorated symptoms in preclinical models of multiple sclerosis, Rett syndrome, Huntington's disease (HD) and Alzheimer's disease (AD). Pepinemab, a humanized anti-SEMA4D monoclonal antibody, was evaluated in a double-blind, randomized, placebo-controlled Phase 2 study in subjects with HD. Subjects were treated with monthly infusions of pepinemab for at least 18 months and evaluated for safety and clinical parameters including cognition (HD-CAB) and Clinical Global Impression of Change (CGIC). Secondary imaging endpoints included structural MRI to assess brain atrophy and FDG-PET to assess brain glucose metabolism. Pepinemab was well-tolerated and was shown to cross the BBB at the anticipated level. Primary efficacy outcomes did not achieve statistical significance, however trends favored pepinemab. Exploratory analyses demonstrated a treatment benefit in 6/6 components of the HD-CAB cognitive battery, with a significant increase in HD-CAB composite index ($p=0.007$) in subjects with early manifest (EM) HD who received pepinemab treatment. Among EM subjects with somewhat more advanced disease progression, treatment also reduced deteriorating CGIC status ($p=0.04$). Pepinemab treatment reduced brain atrophy (volumetric MRI) and slowed or reversed decline in metabolic activity in 26/26 brain regions, with 15/26 ROI showing a significant positive treatment effect ($p\leq0.05$) in FDG-PET imaging. Reversal of metabolic decline associated with disease progression supports the proposed mechanism of restoring astrocytic metabolic homeostasis. Further improvements in cognition highlights the critical role for astrocytes and glia contributing to neuronal function and protection in neurodegenerative processes. SIGNAL-HD demonstrated pepinemab's safety and encouraging treatment effects in a clinical study. The mechanism of action is believed to be applicable to diseases exacerbated by inflammatory glial activation. A Phase 1 study in AD is planned.

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S18-03

White matter microglia activation and cognitive impariment

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Clinically, age related white matter pathology can present as white matter hyperintensities (WMH) on T2-weighted fluid attenuated inversion recovery (FLAIR) MRI, and are associated with an impairment in processing speed, working memory, and are predictive of future cognitive decline, indicating a critical role for white matter integrity in age related cognitive decline. Although the pathophysiology of WMH are heterogeneous, underlying mechanisms pointing to inflammatory processes are a core component of their pathology. Our lab has demonstrated in humans, histologically, that WMH are regions that demonstrate enlarged peri-vascular spaces, blood brain barrier breakdown, axonal degeneration, demyelination and microglia activation. Expanding on this finding in humans, this talk will highlight some of our recent preclinical findings highlighting changes to the white matter in aging and following cerebrovascular stress, stroke and prodromal AD; along with its relationship to cognitive impairment. Using a combination of behavioural testing, focused on executive dysfunction, live animal imaging with PET/MRI

and brain histopathology, we make the case that white matter is a target for therapeutic intervention to preserve cognition during normal aging and early stages of disease progression. Finally, using intervention strategies aimed at reducing microglia activation, we demontrate that targeting white matter activated microglia prevents cognitive impariment post-stroke.

S18-04

Imaging glucose metabolism

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Brain glucose utilization has been widely assessed in clinical settings with fluorine-18 fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) imaging. FDG-PET allows for differential diagnosis in brain disorders, such as Alzheimer's disease (AD). Its biological interpretation assumed for a long time that FDG-PET signal was tightly connected to neuronal activity. However, the dynamics of glucose utilization by brain cells remain not fully understood. A complex interplay between neurons and astrocytes seems to coordinate brain energetics. Indeed, astrocytes take up large amounts of glucose and, thus, it is intuitive that they impact FDG-PET signal. We recently demonstrated that activation of glutamate transport via GLT-1, which is mainly located on astrocytes, acts as a trigger, signaling for glucose uptake in vivo. Our findings provided the first in vivo evidence that FDG-PET signal may indicate more than just neuronal activity, with major implications in its interpretation. In AD, for example, FDG-PET hypometabolism is considered a biomarker of neurodegeneration. However, astrocyte dysfunction may play a substantial role in FDG-PET signal changes occurring in pathological scenarios.

Acknowledgement

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S19 | Studying glia with single cell resolution

(Special Trainee Symposium)

S19-01

Nightlife of the astrocytes: Insights into astrocytic Ca^{2+} signaling in mice during natural sleep

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Astrocytic Ca^{2+} signaling has been intensively studied in health and disease but has not been quantified during natural sleep. Here, we employ an activity-based algorithm to assess astrocytic Ca^{2+} signals in the neocortex of awake and naturally sleeping mice while monitoring neuronal Ca^{2+} activity, brain rhythms and behavior. We show that astrocytic Ca^{2+} signals exhibit distinct features across the sleep-wake cycle and are reduced during sleep compared to wakefulness. Moreover, an increase in astrocytic Ca^{2+} signaling precedes transitions from slow wave sleep to wakefulness, with a peak upon awakening exceeding the levels during whisking and locomotion. Finally, genetic ablation of an important astrocytic Ca^{2+} signaling pathway impairs slow wave sleep and results in an increased number of microarousals, abnormal brain rhythms, and an increased frequency of slow wave sleep state transitions and sleep spindles. Our findings demonstrate an essential role for astrocytic Ca^{2+} signaling in regulating slow wave sleep.

Acknowledgement

Professor Erlend A. Nagelhus tragically died on 10th January 2020. We will always remember him as an inspiring leader, a good friend and a great scientist. We thank Gustavo Borges Moreno e Mello for his contribution to the establishment of the setup for awake animal imaging and monitoring, Arild Njå for guidance on electromyography, Xiaoyi Zhang and Mina Martine Frey for assistance with immunohistochemistry, and Johannes Helm for continuous technical support on the two-photon microscope. We thank Janne Grønli and Jelena Mrdalj for valuable help on establishing rodent sleep scoring. We acknowledge the support by UNINETT Sigma2 AS for making data storage available through NIRD, project NS9021K. This work was supported by the Research Council of Norway (grants #249988, #240476, and #262552), the South-Eastern Norway Regional Health Authority (grant #2016070), the European Union's Seventh Framework Program for research, technological development, and demonstration under grant agreement no. 601055, The National Association of Public Health, The Olav Thon Foundation and the Letten Foundation.

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S19-02

On the discovery of population-specific state transitions from multi-sample multi-condition scRNA-seq data

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Single-cell RNA sequencing (scRNA-seq) has quickly become an empowering technology to profile the transcriptomes of individual cells. A primary tasks for scRNA-seq data is differential expression analysis, which is aimed at finding subpopulation markers by identifying differences between subpopulations.

With the emergence of replicated multi-condition scRNA-seq datasets, an area of increasing focus is making sample-level inferences (i.e. differential state (DS) analysis), termed here as differential state (DS) analysis to investigate subpopulation-specific responses (limited to a single/subset of cell types) of patients measured under different conditions (e.g., healthy/diseased, control/treatment, reference/stimulated etc.). However, it is not clear which statistical framework best handles this situation.

Here, I will present a survey of methods to perform DS analysis, which uses a flexible simulation platform to mimic "complex" scRNA-seq data and systematically evaluate method performances. Finally, I will given an practical example of how DS analysis unravels subpopulation-specific responses within brain cortex tissue, including analysis of astrocytes, oligodendrocytes and microglia, from LPS treated mice.

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S19-03

Neuroinflammatory astrocyte subtypes in the mouse brain

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Astrocytes are the most abundant glial cell type in the central nervous system (CNS) where they perform key homeostatic functions. Depending on which CNS region they occupy, astrocytes can show remarkable transcriptomic and functional heterogeneity. In acute and chronic neuroinflammatory diseases, however, astrocytes undergo an inflammatory transition that can render them dysfunctional and even neurotoxic. Unlike in the healthy brain, we know very little about how heterogenous this inflammatory transition is and how the anatomical location of astrocytes affects their response to an inflammatory insult. To gain better insight into astrocytic heterogeneity in response to inflammation in the mouse brain, we performed single cell RNA-sequencing on ~80.000 FACS-purified astrocytes following injection with either saline or the endotoxin Lipopolysaccharide (LPS). We identify complex astrocytic subtypes that upon LPS injection each show a unique inflammatory response. We also apply spatial transcriptomics (Visium) to pinpoint the anatomical distribution of these astrocytes and uncover that the location of an astrocyte in the brain dictates their response to an inflammatory insult. In particular, we highlight an inflammatory super responder induced by Interferon signaling, that represents less than 2% of all astrocytes, and show that it occupies strategic positions in the brain, including the ventricles, brain surface and penetrating blood vessels. Finally, we show that this super responder is present in a whole range of neurodegenerative diseases but has been missed due to its rarity. Here, we used a multimodal approach to uncover complex astrocytic subtypes and inflammatory substates and highlight their anatomical location in the mouse brain. We also show the power that lies in sequencing large numbers of single cells and use singe cell data set integration with previously published data to extract small but important substates that could otherwise be missed.

S19-04

Spatial Transcriptomics and *In Situ* Sequencing to Study Alzheimer's Disease

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The linear cause-consequence relationship linking amyloid-β peptide (Aβ) accumulation to neuronal dysfunction in Alzheimer disease (AD) is gradually being replaced by the concept that Aβ initiates complex inflammatory-like cellular alterations that progressively become Aβ independent and lead to brain dyshomeostasis. Little is known about the pathophysiology of this cellular phase of AD. Here, we use two orthogonal technologies, Spatial Transcriptomics and in situ sequencing, to analyse the transcriptome changes at the single-cell level in the Aβ plaque niche in a knock-in mouse model of AD. We identify a multicellular co-expressed gene network of 57 Plaque-Induced Genes (PIGs) that define a series of coordinated and spatially restricted microglia, astroglia and oligodendrocyte responses to progressing amyloid plaques encompassing complement, oxidative stress and inflammation. A separate oligodendrocyte network suggests abnormal myelination. Spatial Transcriptomics provides an unprecedented approach to untangle the dysregulated cellular network in the vicinity of pathogenic hallmarks of AD and other brain diseases.

S19-05

Oligodendrocyte heterogeneity during myelin regeneration

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Demyelinating conditions such as Multiple Sclerosis (MS) are characterised by damage to and loss of myelin in the central nervous system (CNS). As no regenerative or neuroprotective therapies currently exist, a major goal is to identify strategies to enhance the regenerative capacity of the CNS. It is well established that remyelination can occur via the generation of new oligodendrocytes from resident-progenitors, but it remains unclear to what extent mature oligodendrocytes, including those that survive demyelination, can contribute to the regeneration of myelin. We use longitudinal live imaging in zebrafish to study myelin regeneration with single cell resolution of both newly generated oligodendrocytes and those that survive demyelination. We show that newly generated oligodendrocytes are far more efficient at regenerating and targeting myelin than those that survive demyelination. We aim to define the molecular and cellular nature of oligodendrocyte heterogeneity during remyelination in zebrafish, and also in humans, through the analysis of MS tissue, in order to identify strategies to promote remyelination for the treatment of human disease.

S19-06

Single nuclei transcriptomic profiling of human astrocytes and oligodendrocytes in Alzheimer's disease

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Growing interest has turned towards the role(s) of astrocytes and oligodendrocytes in Alzheimer's disease (AD). However, little is known about these cell types in human AD patients, such as what characteristics define glia in AD, and how heterogeneous is this response? Here we present the largest-to-date generated single nuclei RNA sequencing (snRNASeq) dataset characterizing astrocytes and oligodendrocytes from human postmortem APOEe2/3 AD and aged-matched non-symptomatic brain. By sorting nuclei based on LHX2+/NeuN- levels, we enrich for astrocytes (5% to 50%) while simultaneously maintaining capture rates of oligodendrocytes (30%) and depleting neurons (<10%). To date, we have sequenced 25,000 oligodendrocytes and 41,000 astrocytes across 14 patients – 30x more astrocytes per donor than previously possible. We identify subtle differences between AD and aged astrocytes and oligodendrocytes, suggesting that age highly influences glia phenotype. This large dataset enables us to integrate smaller snRNASeq datasets for additional power in clustering and ultimately provides a resource to explore astrocyte and oligodendrocyte reactivity in AD and aging.

S20 | Metabolic control of glial identity phenotype and function

S20-01

How lipid metabolism regulates adult neural stem cells

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Neural stem/progenitor cells (NSPCs) generate new neurons throughout life in distinct regions of the mammalian brain and share many features with astrocytes. A tight regulation of NSPC quiescence and proliferation is crucial to ensure life-long neurogenesis and prevent exhaustion or uncontrolled growth of the stem cell pool. What regulates this delicate balance is not fully understood. In my laboratory, we are interested in how cellular metabolism is influencing NSPC activity and how metabolic programs change between quiescent and proliferating NSPCs and their differentiating progeny. I will present our recent findings about the important role of lipid metabolism for NSPCs, how the build-up and breakdown of lipids influences proliferation and quiescence of NSPCs, and how lipid droplets (LDs), the storage organelles of neutral lipids, play a role in this regulation.

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S20-02

Mitochondrial metabolism governs myeloid cell function in the chronically inflamed central nervous system.

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Compelling evidence exists that patients with progressive forms of multiple sclerosis (MS) display pathological changes in neuronal metabolism and mitochondrial function. However, it is unknown if a similar degree of mitochondrial dysfunction occurs in non-neuronal cells in the central nervous system (CNS), and whether it plays causative role in disease progression. Specifically, it remains to be answered (i) whether tissue-resident microglia and infiltrating macrophages undergo mitochondrial structural and metabolic changes after prolonged neuroinflammation, and (ii) whether these alterations underlie a differently pathogenic phenotype that is amenable of therapeutic targeting. Herein we discuss how mitochondrial function and cell metabolism govern the function of chronically activated microglia/macrophages in neuroinflammation, and we identify new metabolic targets for therapeutic approaches aimed at reducing chronic CNS inflammation.

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S20-04

Control of astrocyte heterogeneity in CNS inflammation

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Astrocytes perform multiple roles during CNS inflammation, reflecting their ability to adopt multiple poorly characterized activation states. We have defined several novel astrocyte functions and regulatory mechanisms. We described a novel role for the mitochondrial anti- viral signaling protein (MAVS) in controlling astrocyte pro-inflammatory activities and astrocyte-neuron metabolic interactions, independent of the well-known function of MAVS in anti-viral responses. We also described for the first time the direct regulation of astrocytes by microbial metabolites, and also through a novel pathway involving microglia, VEGF-B and TGFa. Furthermore, we established a unique role for SigmaR1 and the unfolded protein response in controlling astrocyte responses and their modulation by environmental chemicals. Most recently, we identified novel astrocyte activation states with pro- and anti-inflammatory functions controlled by T cells and NK cells, respectively. In this talk, we will review our current knowledge on astrocyte functional diversity and its control in health and disease.

S21 | Sex differences in glia

S21-01

A cell-autonomous pathway that controls sexually dimorphic gene expression in glia

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While sex differences in glia have been observed, their genetic basis remains unclear. From first principles, they could either reflect inherent sex differences in the glia themselves or arise as a response to sex-specific neural environments. We are using a subtype of *C. elegans* glia, called CEPso, as an innovative model to determine the genetic basis of glial sex differences. Although CEPso glia are present in both sexes throughout life, we find that a GFP reporter (*grl-18*pro:GFP) is expressed only in adult male glia, demonstrating that these glia are sexually dimorphic. Interestingly, these glia associate with a neuron that becomes active in adult males, raising the possibility that sexual dimorphism in CEPso glia is a response to the onset of sex-specific neuronal activity. To test this possibility, we genetically manipulated the sex identity of the glia or the neurons. We found that sex-specific expression of the GFP reporter is controlled solely by the sex identity of the glia regardless of the sex identity of neurons, indicating that a cell-autonomous pathway controls sexually dimorphic gene expression in glia. To identify the pathway that controls glial sexual dimorphism, we used candidate and unbiased genetic screens. We found that the established timing factors LEP-2/MKRN and *lep-5* are required to initiate expression of the GFP reporter at the beginning of adulthood and, in mutants lacking these factors, the onset of glial sexual dimorphism is delayed or absent. We also found that GFP reporter expression is absent in adult males lacking the established sex identity factor MAB-3/DMRT while, conversely, forced expression of MAB-3 in CEPso glia of juveniles or in the opposite sex induces inappropriate expression of the GFP reporter, suggesting that MAB-3 activity in glia is necessary and sufficient for sexually dimorphic gene expression. Finally, we identified the transcriptional repressor NFYA-1/NF-Y as a factor that prevents expression of the adult male GFP reporter in glia of the opposite sex. Our results lead to a model in which sex-specific expression of MAB-3 in CEPso glia represses NFYA-1 to permit expression of adult male-specific glial genes. This study defines a pathway by which glia control their own sexually dimorphic gene expression, and suggests that glia are ideally positioned to create – and not merely respond to – a sex-specific neural environment.

S21-02

Microglia sexual differentiation

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It is well known that the sex has a major relevance in the incidence of pathologies affecting the immune system, but the real nature of this phenomenon remains to be elucidated. Considering the major role of neuroinflammation in

the onset and progression of several neurological diseases, we decided to evaluate the activity of microglia in males and females adult mice. For this study, our laboratory developed several tools, including cellular and animal models, which enabled in-depth studies on sexual differentiation of microglia and its impact on brain physiology, as well as on the onset and progression of neurological disorders. We will describe the significant differences found in the transcriptome of adult male and female microglia. Interestingly, microglia isolated from adult brains was show to maintain the sex-specific features when grown in culture or transplanted in the brain of the opposite sex. This suggests a limited functional effect of sex steroids in microglia activities and the potential developmental effect of perinatal exposure to sex steroids. Overall, female microglia was shown to be neuroprotective in case of acute injury. Our study therefore provides a novel insight of the mechanisms underscoring a sexual bias in the susceptibility to brain diseases. The role of sexual differentiation of microglia in neurodegenerative diseases will be discussed.

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S21-03

Microglia developmentally regulate astrocyte and neuron number creating enduring sex-specific behavioral changes

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Microglia are important purveyors of brain maturation via their capacity to both prune and promote the formation of synapses. Equally important but less well understood is the ability of microglia to actively engulf and subsequently kill specific target cells, thereby controlling population density. We have reported that within the developing medial amygdala (MeA), microglia specifically target astrocyte progenitors for phagocytosis, and, they do so more in the male brain than the female brain (VanRyzin et al, Neuron 2019). This androgen-driven process occurs during the perinatal period and results in a reduced astrocyte population within the juvenile MeA. The MeA is a known critical node in the neural circuitry controlling juvenile social play. The lower astrocyte density in the MeA of young males correlates with increased neuronal activation while engaged in rough-and-tumble play, a behavior more robustly expressed by males. Coaxing neonatal female microglia to increase phagocytosis of astrocyte progenitors recapitulates the same process normally seen in males, including increased playfulness as juveniles whereas blocking microglia phagocytosis in males has the opposite effect, thereby confirming that early life programming of astrocyte density by microglia establishes later life behavioral sex differences. A parallel but distinct process occurs within a subnucleus of the medial preoptic area called the sexually dimorphic nucleus (SDN), named for its feature of being substantially larger in males than females. The function of this small collection of Nissl-dense neurons appears to be regulation of sexual partner preference, with a larger SDN conferring preference for females and a smaller one, a preference for males. The developmental origins of the differential size of the SDN has long been believed to be the result of increased apoptosis in females due to lack of androgen-derived trophic or survival factors. We have now determined this is not the case. Rather, microglia in and around the SDN are more phagocytic in females and they are engulfing and killing the maturing Nissl-dense neurons. Preventing this process

with blocking antibodies in females results in a male sized SDN and a switch in odor preference to that of typical developing males. Thus microglia are essential determinants of sex-specific establishment of multiple neuronal

circuits regulating complex social behaviors.

S21-04

Sexual dimorphism of microglia molecular states

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Investigating sex differences of microglia has been done using several methods. In the pioneering papers density and morphology was used. Later, microglia function such as phagocytosis and response to injury were studied. Some papers have analyzed the transcriptome of microglia freshly isolated from homeostatic and diseased brains. We here aim to compare transcriptomic signatures from male and female microglia published in the recent years. In addition we investigate the impact of different protocols and methods on the differences in these microglia states. We hope to contribute to a better standardisation of procedures for microglia isolation, RNA and libary preparation as well as bioinformatic analysis. This will help to make the studies from different laboratories to be more comparable and identify good targets for personalized medical research.

S22 | Understanding the role of glia in neurodevelopmental disorders using pluripotent stem cell-derived human glia

S22-01

Modeling neurodevelopmental disorders using induced astrocytes

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Astrocytes have over the last decades emerged as key players in neurological disease. However, due to scarcity of human native astrocytes and differences in model organisms' counterparts it remains largely unknown through which mechanisms and to what extent they act during disease. Development of stem cell technologies and generation of human induced pluripotent stem cells has allowed for studies of human cell types previously not available. Astrocytes develop late during embryogenesis and traditional methods of differentiating human pluripotent stem cells (hPSC) using external cues to mimic development thus requires months of cell culture to obtain functional and mature astrocytes. Contrarily, ectopic overexpression of cell linage specific transcription factors enables to fast forward this process.

We recently developed a rapid transcription factor driven method to generate astrocytes from hPSCs. We showed that overexpression of the gliogenic transcription factors Sox9 and Nfib in hPSCs yield a highly homogenous population of astrocytes as early as 7 days post-transduction. After 14 days, these induced astrocytes (iAs) exhibit molecular signatures and functional properties closely resembling those of adult human astrocytes. iAs have the capacity for neurotransmitter clearing, form gap junctions, display spontaneous and evoked calcium waves, are immunocompetent and provide neurotropic support for human induced neurons. By combining our method with CRISPR/Cas9 genome-engineering we demonstrated that iAs can be used to model and reveal pathological astrocytic phenotypes in Alexander disease (Canals et al., 2018).

Furthermore, we have been able to directly convert human fibroblasts to astrocytes. This is an important complementary approach that has the potential to better model late onset disorders and allows to dissect epigenetic components of disease mechanisms.

We are currently using our methods for generating human astrocytes in combination with patient fibroblasts, iPS cells and CRISPR genome engineering to model leukodystrophies and early childhood dementia.

Our methods allow for rapid and easy generation of patient specific astrocytes and enable investigation of human astrocyte biology in health and disease, astrocyte-neuron interactions and have the potential to uncover astrocyte-associated disease mechanisms.

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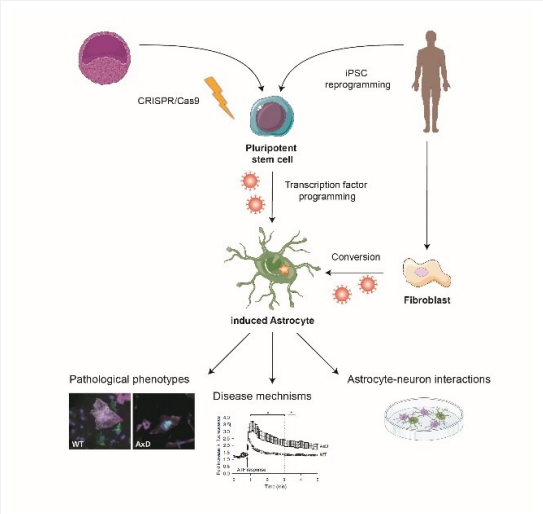

Transcription factor driven methods to generate human astrocytes for disease modeling
We have developed methods for generation of human induced astrocytes using ectopic expression of transcription factors. We combine these with patient fibroblasts, iPS cells and CRISPR genome engineering to model neurological disorders.

Adapted from Canals et al., 2018, Nature Publishing Group

S22-02

Suppresion of PLP1 for Pelizaeus Merzbacher Disease

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Pelizaeus-Merzbacher disease (PMD) is a monogenic pediatric X-linked leukodystrophy that is caused by a variety of mutations in the proteolipid protein 1 (*PLP1*) gene. Hundreds of mutations have been identified in humans, and these mutations result in a spectrum of clinical severities. Supernumerary copy variants and point mutations often result in severe disease and early mortality. In a severe conatal point mutation mouse model of PMD we previously demonstrated that treatment with *Plp1* targeting antisense oligonucleotides (ASOs) stably suppressed *Plp1* throughout the neuraxis, rescued cellular and behavioral deficits, and extended lifespan from 21 days to over 14 months. To translate this strategy to humans, we generated 3D oligocortical spheroids from human induced pluripotent stem cells from PMD patients with either a *PLP1* duplication or a point mutation resulting in severe disease. The human PMD oligocortical spheroids exhibited deficits in oligodendrocyte numbers and morphology consistent with the hypomyelination seen in patients. Treatment of human PMD oligocortical spheroids with PLP1 targeting ASOs reduced *PLP1* levels and rescued oligodendrocyte deficits. Collectively, these results in PMD mice and human PMD oligocortical spheroids demonstrate the potential utility of oligonucleotide therapeutics as a promising strategy for the treatment of PMD.

S22-03

Modeling Neurodevelopmental Disorder Using Human iPSC-Based Microglial Mouse Chimeras

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Microglia, the brain-resident macrophages, play critical roles in maintenance of brain homeostasis and regulation of diverse neuronal responses, such as neurogenesis, neural differentiation, and synaptic development. Mounting evidence indicates that rodent microglia are not able to fully mirror the pathophysiology of human microglia. Transcriptomic profiling of human and mouse microglia reveals species-specific expression patterns in genes involved in brain development, immune function, and phagocytic function. Recent advances in stem cell technology have led to the efficient generation of microglia from human induced pluripotent stem cells (hiPSCs), providing an unlimited source of human microglia to study their function. Down syndrome (DS), caused by triplication of human chromosome 21 (Hsa21), is the most common genetic cause of intellectual disability and developmental delay, and affects one in every 700-800 live births. Little information is available on how trisomy of Has21 alters the development and functions of microglia and what roles microglia play in the abnormal brain development and cognitive deficits in DS. We recently developed novel cerebral organoid and human-mouse chimeric brain models that contain human hiPSC-derived microglia. Those models provide new opportunities to better understand the biology of human microglia and their role in the pathogenesis of DS.

S22-04

Human astrocytes in neurological diseases

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Astrocytes are an integral part of the neural networks and are involved in the pathogenesis of numerous neurological and psychiatric conditions. However, the specific roles of astrocytes in the pathogenesis of neurological disorders are not clear. We have developed means to guide human pluripotent stem cells to regionally and functionally diversified astroglial subtypes. These cells retain the unique features of human astrocytes in vitro and following transplantation into the rodent brain and spinal cord. Using astrocytes derived from patient induced pluripotent stem cells (iPSCs), including those from Alexander disease (caused by mutations in GFAP) patients, I will discuss roles of GFAP in astrocyte functions and the bi-directional interactions between human astrocytes and human neurons in neurodegeneration.

Acknowledgement
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S23 | Mitochondrial regulation of astrocyte function in physiological and pathological conditions

S23-01

Mitochondrial structure and calcium dynamics in astrocytes

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The transient opening of the mitochondrial permeability transition pore induces spatially restricted Ca^{2+} transients in astrocyte processes, providing a means to link astrocyte respiration rates and Ca^{2+} dependent effector pathways. However, the cross-talk between cytosolic and mitochondrial Ca^{2+} signals in astrocytes still remains elusive. To simultaneously record and characterize cytosolic and mitochondrial Ca^{2+} dynamics, we have developed novel transgenic mouse lines and AAV-based viral approaches to express various fluorescent proteins as well as genetically encoded Ca^{2+} indicators in various astrocytic compartments. Additionally, to automatically segment mitochondria and study the structural and Ca^{2+}dynamics, we developed a machine-learning-based algorithm called mito-CaSCaDe. Using 2-photon microscopy-based Ca^{2+} imaging, we found that mitochondria exhibit spontaneous fluctuations in matrix Ca^{2+} and activation of astrocytes by neuromodulators such as norepinephrine induced long-lasting Ca^{2+} transients in mitochondria. Using our mouse genetics tools, optical and serial-section scanning electron microscopic analysis, we discovered that mitochondria in astrocytes formed densely networked structures and were not very motile. In this symposium, I will discuss the new results that are helping us to decipher the role of astrocyte Ca^{2+} signals in the cytosol and mitochondria in shaping astrocyte functions in the brain.

S23-02

Mitochondrial network dynamics in reactive astrocytes

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Following brain injury, astrocytes acquire reactivity states which underlie important functions in the progression of the injury and its possible resolution. Using a combination of imaging, genetic and omics approaches we investigated the structural and functional changes in organelle networks experienced by astrocytes reacting to injury and blood-brain-barrier disruption *in vivo*. We found that a marked remodelling of the mitochondrial network underlies the generation of a spatially-defined mitochondrial-ER enriched domain in astrocytic perivascular end-feet. Manipulation of mitochondria-ER tethering in astrocytes revealed the significance of this response in

sustaining angiogenesis at the injured site. These results establish an important mechanism for astrocytic mitochondrial dynamics in orchestrating cellular metabolic adaptations *in vivo* and unravel a key role for astrocytes in sustaining microvasculature remodelling during repair.

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S23-03

Mitochondrial biogenesis in developing astrocytes regulates astrocyte maturation and synapse formation: implication for 22q11 deletion syndrome

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One of the main candidate risk gene for behavioural phenotype of 22q11 deletions syndrome (22q11DS) is the gene encoding for proline dehydrogenase (PRODH), a mitochondrial enzyme involved in the metabolism of L-proline. The expression of PRODH is typically ascribed to proliferating tissues (Polyak et al., Nature, 1997) where it regulates cell proliferation (Donald et al., Cancer Res, 2001) and metabolism (Liu et al., PNAS, 2012). The functional role of PRODH in the brain is not completely understood. Here, we found that PRODH expression in the brain is particularly enriched in astrocytes during postnatal development where it regulates the levels of a metabolic regulator PPARγ co-activator 1α (PGC-1α). Interestingly, the transient increase of PGC-1α in developing astrocytes is necessary to induce astrocytic mitochondrial biogenesis and to co-ordinate postnatal astrocyte maturation and synaptogenesis. Analysis on astrocytes of PRODH deficient mice (Paterlini et al., Nature Neurosci., 2005) have shown that the transient increase of PGC-1α in developing astrocytes is perturbed and both the respiratory capacity and the ATP levels were significantly impaired thus suggesting that PRODH expression is necessary to maintain a proper mitochondrial function. Together with the finding of the development-associated enhancement of mitochondrial function in astrocytes, our results uncover mitochondrial biogenesis as a novel mechanism controlling astrocyte morphogenesis and supporting synaptogenesis, thus suggesting that astrocytic mitochondria may be a therapeutic target in the case of neurodevelopmental and psychiatric disorders characterized by impaired synaptogenesis

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S23-04

Astrocytic Mitochondrial Dysfunctions During Dementia

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A typical feature of dementia (including Alzheimer's disease) is the accumulation of the tau protein in neurons, neurodegeneration and the loss of hippocampal-dependent memory. However, the role of non-neuronal cells in this chain of events remains unclear. We found evidence of the accumulation of the 3R isoform of tau in hilar astrocytes of the dentate gyrus of patients. Furthermore, Alzheimer's disease severity was associated with the proportion of astrocytes accumulating the 3R but not with astrocytes accumulating the 4R isoform of tau in the hilus. Using a novel viral tool to specifically target astrocytes of the hilus, we found that accumulation of the human wild-type 1N3R isoform of tau in these cells strongly impaired mitochondrial motility, distribution and functions in distal processes, whereas 1N4R induced less drastic modifications. In turn, 1N3R overexpression resulted in several neuronal impairments in the dentate gyrus, including impaired adult neurogenesis, a reduction in the number of neurons expressing the activity-dependent calcium-binding protein parvalbumin, a decreased density of inhibitory synapses, and a reduced gamma oscillatory activity in dentate gyrus. Together, these modifications led to impaired spatial memory, which was restored upon stimulating PV interneuron activity.

These results indicate that mitochondrial functions in astrocytes (in particular in their distal processes) are vital for the physiology of the hippocampus and for mechanisms of learning and memory.

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S24 | Glia-axonal communication in nervous system injury and disease

S24-01

Do injured axons signal to Schwann cells prior to axon degeneration?

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Myelinating and non-myelinating Schwann cells undergo a radical change of cellular state after nerve injury, forming repair Schwann cells [1]. These cells develop an elongated branched morphology, are specialized for clearing myelin debris, using myelinophagy, supporting axonal regeneration and maintaining survival of injured neurons [2, 3, 4]. This process is regulated by a number of Schwann cell-intrinsic signals, however, little is currently known about the extent to which extrinsic signals from axons may instruct Schwann cells to become repair cells?

In the mouse injured nerve, axonal degeneration of large myelinated fibres, in sites remote from the injury, has been said to occur between 36-42 hours after nerve transection [5]. Many prior nerve injury studies in rodents have shown potential Schwann cell gene and protein expression changes at very early time points, however it has been unclear whether these changes only take place near to the site of injury, where axons immediately break down and there is large scale tissue inflammation.

To address the question of whether Schwann cells biochemically react to nerve injury prior to axon degeneration, we have performed RNA sequencing of the injured tibial nerve at an anatomical site very remote from the initial transection (sciatic notch), at several time points prior to the point of axon degeneration. We have asked if early injury gene expression changes rely on the intrinsic molecular machinery of axon degeneration, through activation of Sterile- α and Toll/interleukin 1receptor (TIR) motif containing protein 1 (SARM1)? Our results will be discussed within the wider context of whether they refute or support the existence of substantial axon injury signalling to Schwann cells.

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S24-02

Probing cellular and molecular mechanisms of peripheral nerve regeneration using zebrafish

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Unlike axons of the central nervous system, axons of the peripheral nervous system have retained the capacity to remake functional synapses even after complete nerve transection. Yet despite their unique ability to re-make functional connections, we know remarkably little about how peripheral axons, such as motor axons re-connect with their original muscle targets. This is in part because the dynamic behavior of injured axons as they respond to insults, interact with neighboring Schwann cells, and begin to pioneer a path towards their original targets, has not been examined in real time, in intact vertebrate animals. We have established a laser based nerve transection model in zebrafish, enabling us to visualize the cellular behaviors of transected axons and neighboring Schwann cells simultaneously, in real time, in an intact vertebrate animal (1, 2). Using this model we screened a library of existing zebrafish mutant lines to identify molecular entry points into the process of peripheral nerve regeneration (3, 4). I will discuss progress on ongoing projects aimed to understand the cellular and molecular mechanism that promote peripheral nerve regeneration.

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S24-03

Exploring transcriptional responses that govern glial immune responses to injury in *Drosophila*.

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Glial cells are highly sensitive to any form of trauma, including acute neural injury, and pathogenic insults, as well as chronic neurodegenerative conditions. Responding glia display striking changes in immune gene transcription and morphology to access trauma sites, secrete protective factors, and clear damaged cells through phagocytosis. However, the precise molecules and signaling pathways that drive glial immune reactions to neuronal damage are still unclear. Understanding how glial responses are activated, carried out, and ultimately quenched will offer new

insight into methods that could enhance neuroprotection following injury and delay the onset and progression of a range of neurodegenerative disorders. The fruit fly *Drosophila melanogaster* is a powerful genetic model system to study *in vivo* neuron-glia signaling events in the damaged or diseased brain, as well as conserved glial immune responses. Our lab has recently shown that the secreted protease matrix metalloproteinase-1 (Mmp-1) is robustly upregulated in ensheathing glial cells after axon injury and required for proper glial clearance of axonal projections. We hypothesize that Mmp-1 is secreted from glial extensions at sites of injury and wondered how mmp-1 transcript localization might contribute to the timely production and release of this key protease. We are currently modifying novel techniques for the detection of individual transcripts (single molecule fluorescence in situ hybridization, smFISH) and their association with ribosomes (fluorescence assay to detect ribosome interactions with mRNA, FLARIM). Interestingly, our preliminary findings suggest that mmp-1 transcripts are upregulated specifically within responding ensheathing glial cells after injury and distributed along lengthy glial processes at sites where glia contact axons. Our findings also suggest that mmp-1 transcripts are translated in distal glial processes in response to axotomy. In the future, we hope to explore how directed RNA transport and local translation of key immune genes contribute to proper innate glial immune signaling mechanisms in the adult brain after trauma.

S24-04

Neuregulin-1 signaling in repair responses of peripheral nerve diseases

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The peripheral nervous system harbors a remarkable potential to repair after acute nerve injury. We could previously show that denervated Schwann cells after acute nerve injury induce the de-novo expression of soluble Neuregulin-1 (NRG1), a timely limited signal that promotes nerve repair and remyelination. In contrast to acute injury, the molecular response of Schwann cells in chronic neuropathies is poorly understood. Onion bulb structures are a pathological hallmark of demyelinating neuropathies, but the nature of these formations remained unknown. We could demonstrate that Schwann cells induce the expression of Neuregulin-1 type I also in various chronic demyelinating diseases. Genetic disruption of Schwann cell-derived NRG1 signaling in a mouse model of Charcot-Marie-Tooth Disease 1A (CMT1A) resulted in a pronounced amelioration of the clinical disease phenotype along with a suppression of key histological disease hallmarks including the formation of onion bulbs. Transgenic overexpression of NRG1-I in Schwann cells on a wildtype background was sufficient to mediate an interaction between Schwann cells via an ErbB2 receptor-MEK/ERK signaling axis, causing onion bulb formations and a peripheral neuropathy reminiscent of CMT1A. We suggest that diseased Schwann cells mount a regeneration program that that is beneficial in acute nerve injury, but that overstimulation of Schwann cells in chronic neuropathies is detrimental.

S25 | Satellite glial cells – unique glia of the peripheral nervous system

S25-01

Recent insight into the SGC transcriptional injury response

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Satellite glial cells (SGCs) are homeostatic cells enveloping the somata of peripheral sensory and autonomic neurons. A wide variety of neuronal stressors including injury, disease or drug treatment trigger activation of SGCs, which is believed to modulate neuronal activity of relevance for neuropathic pain and/or a regenerative response. However, compared to neurons and other glial cells, SGCs have received modest scientific attention and very little is known about SGC biology. This is, in part, due to the technical challenges of correctly identifying and manipulating them *in vivo* owing to their very flattened structure and close physical proximity to neurons. Attempts have been made to study them *in vitro*, but this approach has shortcomings as cultured SGCs display significant alterations in phenotype and protein expression initiated after a few days in culture.
The availably of single cell analysis now offers an opportunity to study the transcriptional response of SGC from *in vivo* models. This talk will address how such transcriptional studies can contribute to an increased understanding of SGCs in health and disease.

S25-02

The pharmacology of satellite glial cells

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Satellite glial cells (SGCs) are unique among glial cells in many ways. They reside in sensory/sympathetic ganglia, where they are in an uncommon close relationship with neurons. In fact, they are tightly connected to one another and constitute a protective sheath that wraps around neuronal cell body. Thanks to this specific anatomical localization, they signal to and receive signals from neurons, contributing to the modulation of neuronal firing in both physiological and pathological conditions. In the last 15 years, several studies have started to identify the most important signaling systems that are involved in neuron-to-SGC but also in SGC-SGC communication. In this respect, a prominent role is played by the purinergic system and by gap junctions, respectively. Concerning purines, both P2X/P2Y nucleotide and P1 adenosine receptors have been identified, and alterations of their

expression patterns have been observed upon painful conditions. Additionally, agents acting selectively at some of these receptors (e.g., the ionotropic P2X7, which is also connected to pannexin 1 channel, and the metabotropic $P2Y_2$ subtypes) have demonstrated analgesic actions in rodent model of trigeminal and neuropathic pain [Magni & Ceruti, 2014; Magni et al., 2015]. SGCs are also sensitive to endothelin and express guanylate cyclase which produces cGMP upon neuronal-derived NO stimulation. Moreover, SGCs represent the interface between blood-derived substances and neurons, likely contributing to convey signals from the circulation to the peripheral and, ultimately, the central nervous system [Hanani & Verkhratsky, 2021]. Pathological conditions have been demonstrated to modify the pattern of communication among SGCs themselves, with the upregulation of specific connexins to strengthen calcium-mediated signaling which in turn might impact on neuronal firing. Although additional studies are needed to dissect the whole cell-to-cell communication network within peripheral ganglia, available data clearly highlight the fundamental role of SGC in physiology and pathology and suggest that they represent yet-to-be fully exploited pharmacological targets for innovative therapies against several pathologies, including neuropathic and inflammatory pain.

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S25-03

Neuron-satellite glia interactions and pain

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The main sources of sensations from the body are neurons in the dorsal root ganglia and trigeminal ganglia. These sensory ganglia relay various sensations, including pain, to the central nervous system (CNS). Following peripheral injury or inflammation, neurons in these ganglia fire abnormally, and are therefore a major source of nociceptive signals. The main type of glial cells in sensory ganglia are satellite glial cells (SGCs). These cells completely surround cell bodies of neurons with a space of only 20 nm, which allows close bidirectional SGC-neuron interactions (Fig. 1). This organization of a well defined neuron-SGC unit is unique to peripheral ganglia, and not found in the CNS. SGCs in sensory ganglia are activated by numerous types of nerve injury and inflammation. The activation includes upregulation of glial fibrillary acidic protein, increased gap junction-mediated SGC-SGC and neuron-SGC coupling, augmented sensitivity to ATP via P2 receptors, downregulation of Kir4.1 potassium channels, and enhanced cytokine synthesis and release. These changes in SGCs lead to augmented neuronal activity, and thus contribute to acute and chronic pain. We proposed that the combination of augmented gap junctional coupling with greater P2 receptor responses contribute to stronger calcium waves in the ganglia, leading

to greater neuronal excitability. Indeed, blocking gap junctions reduced pain behavior in somatic and orofacial pain models in rodents, indicating the important role of gap junctions in nociception. It appears that a major factor in SGC activation is the release of nitric oxide from neurons, which diffuses to SGCs, and stimulates cGMP synthesis in them. SGC activation was observed in numerous animal models of pain, including local and systemic inflammation, various types of surgery, diabetic neuropathy, axotomy, and anti-cancer drugs such as oxaliplatin. In all these models, the changes in SGCs were very similar, indicating that SGC activation is a general feature in pain states. Therefore, a better understanding of these changes and the resulting abnormal interactions of SGCs with sensory neurons could lead to novel approaches to alleviate and prevent pain. Moreover, SGCs have a potential to be a target for pain therapy because sensory ganglia do not have a vascular barrier and are therefore exposed to the circulation.

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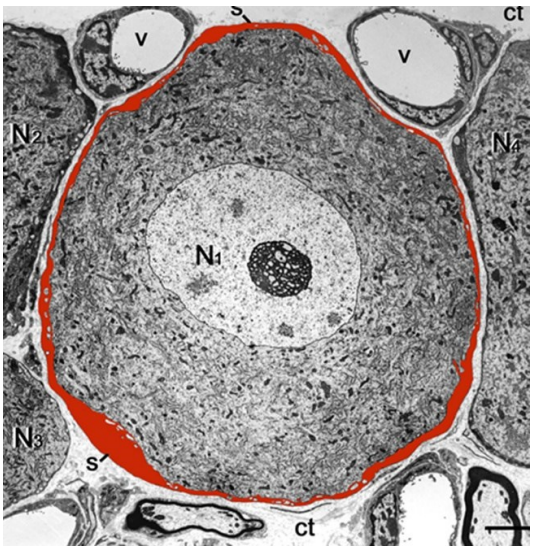

Fig. 1. The organization of the satellite glial cell (SGC)-neuron unit.
A low power electron microscopic image of a mouse dorsal root ganglion. Neurons (N1-4) are surrounded by SGCs. The SGC sheath around N1 is marked red. s-SGC, v-blood vessel, ct-connective tissue space. Scale bar, 4 µm. Modified from Ledda et al., Neuroscience 164 (2009) 1538–1545

S25-04

Diversity of satellite glia in sympathetic and sensory ganglia

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Satellite glia are the major glial type found in ganglia of the peripheral nervous system that wrap tightly around cell bodies of sympathetic and sensory neurons that are remarkably diverse. Despite the close physical association of satellite glia with peripheral neurons, little is known about this glial population. Using single cell RNA sequencing analysis, we identify considerable diversity of satellite glia in sympathetic and sensory ganglia that suggest ganglia-specific functions. We also use genetic ablation studies in mice to define roles for satellite glia in the adult sympathetic nervous system in providing trophic support to neurons and for regulating neuronal activity, in part, via potassium buffering.

S26 | Innate immunity and microglia

S26-01

Microglial opsonins, chaperones and alarmins

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Microglia mediate their physiology, pathology and innate immunity in part via releasing and responding to opsonins, chaperones and alarmins.

Opsonins are normally soluble, extracellular proteins, which when bound to cells induce phagocytes to phagocytose such opsonin-tagged cells. Opsonins do this by binding: i) either PAMPs on pathogens, DAMPs on debris or 'eat-me' signals on self cells, and ii) phagocytotic receptors on phagocytes, to induce engulfment. Microglia do not normally phagocytose anything without opsonin tagging. Activated microglia release multiple opsonins, including MFG-E8, Gas6, C1q and APOE. We provide evidence that activated microglia release calreticulin and galectin-3 to opsinise bacteria, synapses and neurons, via binding desialylated glycans on these targets, and to LRP1 or MerTK on microglia.

Alarmins are soluble factors released by cells to activate an immune response in other cells. Evidence is presented that activated microglia and other cells release calreticulin and galectin-3, and these act as alarmins attracting and activating microglia.

Extracellular chaperones refold or prevent misfolding of proteins. Evidence will be presented that activated microglia release sTREM2 and calreticulin, which block amyloid pathology by acting as chaperones, while R47H sTREM2 and galectin-3 increase amyloid pathology partly by promoting misfolding of beta amyloid.

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S26-02

Immune memory and microglial phagocytosis

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It has become clear that macrophages (including microglia in the brain) are capable of immune memory, i.e. their responses are modified by prior inflammatory insults, even many months in the past. This change in microglial responsiveness is driven by epigenetic reprogramming and affects (amongst other things) their phagocytic activity. In mouse models of Alzheimer's pathology, microglial immune memory states alter their uptake of aggregated amyloid-β and change how they interact with amyloid plaques. Such long-term modification of microglial responsiveness may affect the progression and severity of Alzheimer's disease pathology.

S26-03

Intact sialylation prevents complement C3-mediated loss of neurons in the brain of mice

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Sialic acids form the terminal cap of glyco-proteins and glyco-lipids on the surface of mammalian cells. Sialylation is highly prevalent in the central nervous, as well as the immune system. Recently, it was recognized that sialylation acts as a checkpoint for innate immune responses in the central nervous system. Particularly, sialylation is sensed by sialic acid-binding Ig-like lectin (Siglec) receptors that inhibit the oxidative burst of phagocytes and recognized by complement factors to inhibit the activation of the complement system.
Mice heterozygous for the sialic acid biosynthesis enzyme glucosamine-2-epimerase/N-acetylmannosamine kinase (GNE+/-) show slightly reduced sialylation in different brain regions. Interestingly, hyposialylation was mostly visible by reduced staining for polysialylated neural cell adhesion molecule (NCAM). At 6 months of age these GNE+/- mice had less synapses in the hippocampus, as determined by colocalized puncta that were stained with antibodies against vGlut1 and PSD95. Furthermore, GNE+/- mice showed reduced microglial arborization with reduced tree length and less branches, as well as less junctions and tips. At 12 months of age, GNE+/- mice also had increased loss of neurons in the substantia nigra and the CA3 region of the hippocampus. Data indicate that a homeostatic phagocytic process was leading to the removal of synapses and neurons in aging GNE+/- mice.

Interestingly, deficiency of the complement factor C3 fully rescued the loss of neurons and synapses, as well as the reduced microglial arborization.

Thus, intact sialylation of the brain acts as a checkpoint to prevent complement factor 3 and most probably microglial-mediated removal of synapses and neurons.

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S26-04

Phagocytosis of apoptotic cells: the light at the end of the tunnel for microglia

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From development to aging and disease, the brain parenchyma is under the constant threat of debris accumulation, in the form of dead cells and protein aggregates. To prevent garbage buildup, the brain is equipped with efficient phagocytes: the microglia. However, phagocytosis is not simply the process of debris removal and in this talk I will discuss that it is in fact the beginning of a new life for microglia. Phagocytosis remodels microglia at the metabolic, transcriptional and epigenetic levels, resulting in long-term functional alterations that impact on the surrounding neurons. Among these changes, we have further explored the role of the phagocytosis secretome on the adult hippocampal neurogenic cascade, where microglia efficiently remove the excess of newborn cells. In physiological conditions, phagocytosis is very efficient and the phagocytosis secretome supports the long-term maintenance of adult hippocampal neurogenesis. In contrast, phagocytosis is impaired in mouse models of epilepsy (kainate administration, cystatin B knock-out) as a result of neuronal hyperactivity, as well as in biopsy and autopsy tissue from epileptic patients. Our data suggests that promoting microglial phagocytosis in the epileptic brain may go beyond removing apoptotic debris to actively promoting brain regeneration.

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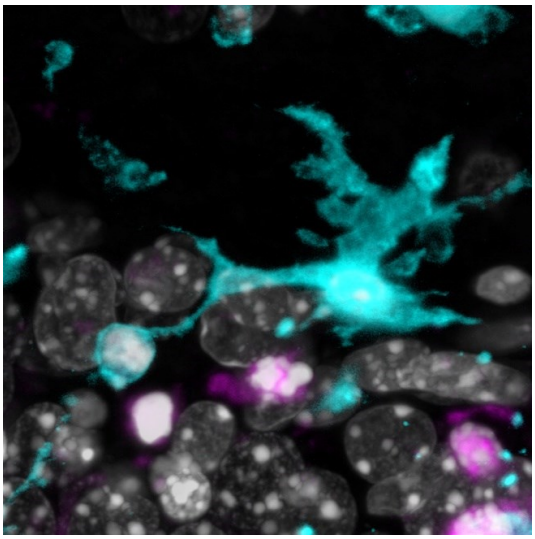

Microglial phagocytosis of apoptotic cells
Confocal 3D reconstruction of a microglia (fms-EGFP+, cyan) engulfing an apoptotic cell (activated caspase 3+, magenta) in the neurogenic niche of the hippocampus (Abiega et al., PLoS Biol 2016). Healthy and apoptotic (pyknotic) nuclei are labeled with DAPI.

S27 | Emerging pathways and therapies to enhance Repair Schwann cells functions

S27-01

Different cell types cooperate with repair Schwann cells to promote nerve regeneration inside a hollow conduit used to repair a nerve gap.

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The repair of a severe nerve injury requires the use of an autograft or a nerve guidance conduit to bridge the gap and avoid axon dispersion and off target reinnervation. Different conduits are routinely used in clinical practice, but their efficacy is comparable with autograft only for short gaps. Understanding the nerve regeneration within hollow conduits might improve their design and their luminal enrichment to reach a good efficiency also for longer gaps.

A nerve guidance conduit is an environment completely different from both the autograft and the distal portion of the nerve, where Wallerian degeneration occurs and most of the players involved in the nerve regeneration are already on site. The empty conduits need to be colonized by different cell populations: nerve fibroblasts, endothelial cells, repair Schwann cells and macrophages. The regeneration within conduits was investigated in the past at morphological level by means of electron microscopy analysis of silicon nerve guidance conduits [1-3].

We decided to further investigate the nerve regeneration within a conduit using different approaches, both at biomolecular and morphological level. To this aim, 10 mm rat median nerve gaps were repaired with chitosan conduits and the nerves regenerated within the conduits were analysed at different time points.

At biomolecular level we analysed the expression of soluble Neuregulin1, a factor involved in Schwann cell trans-differentiation, and the expression of markers of different cell populations, including fibroblasts, endothelial cells, repair Schwann cells and macrophages, and the results were compared with those of the autograft and of the distal portion of regenerating nerves. Interestingly, we identified nerve fibroblasts as a possible source of soluble Neuregulin1, together with Schwann cells[4].

Moreover, by immunofluorescence analysis, we observed that migrating Schwann cells use newly regenerated blood vessels as a substrate for migration within the conduit, a phenomenon previously shown by others within the "nerve bridge" model - the nerve tissue spontaneously regenerated after a nerve cut[5,6] – but not yet shown within nerve guidance conduits.These results reinforce the idea that angiogenesis within nerve conduits plays a key role in nerve regeneration, not only to sustain cell survival, but also to provide a path for migrating repair Schwann cells, thus suggesting that promoting vascularization[7] might be a good strategy to enhance nerve regeneration within longer conduits.

Acknowledgement

Reaxon® Chitosan Nerve Guides were supplied by Medovent GmbH (Mainz, Germany).

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S27-02

The well documented failures of nerve repair seen during aging and chronic denervation involve reduced Schwann cell c-Jun, and are rescued by restoring c-Jun levels.

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Background. After nerve injury, myelin and Remak Schwann cells reprogram to repair Schwann cells specialized for regeneration. These cells activate myelinophagy to break down myelin sheaths and up-regulate cytokine expression to attract macrophages that help myelin clearance. They activate expression of trophic factors that have a major role in repressing death of injured neurons. Repair cells also undergo striking elongation and branching to form compact cellular columns, Bunger bands, that guide axons back to their targets. Although these cells normally provide strong regenerative support, they fail in two important situations: in aging animals, and during the chronic denervation that inevitably results from the slow rate of axonal regeneration. This impairs axonal regeneration and causes significant clinical problems. Previously, we showed that the transcription factor c-Jun is elevated 80-100-fold in Schwann cells of injured nerves and that this factor acts as a strong global amplifier of the regeneration-supportive phenotype of repair cells (Arthur-Farraj et al.2012; Jessen and Mirsky 2019). **Results.** In the present work, we show that c-Jun is central to the regeneration failures seen during aging and chronic denervation (Wagstaff et al. 2021). In mice, we find that repair cells express reduced c-Jun protein as regenerative support

provided by these cells declines both during aging and chronic denervation. In both cases, genetically restoring Schwann cell c-Jun levels is sufficient to restore regeneration to control levels. We identify potential gene candidates mediating this effect. In particular, we implicate Shh in the control of Schwann cell c-Jun levels. **Conclusions.** This establishes that a common mechanism, reduced c-Jun in Schwann cells, regulates success and failure of nerve repair both during aging and chronic denervation. These experiments provide a molecular framework for addressing important clinical problems, suggesting molecular pathways that can be targeted to promote repair in the PNS.

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S27-03

YAP and TAZ regulate Schwann cell proliferation and differentiation during peripheral nerve regeneration

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YAP and TAZ are effectors of the Hippo pathway that controls multicellular development by integrating chemical and mechanical signals. Peripheral nervous system development depends on the Hippo pathway. We previously showed that loss of YAP and TAZ impairs the development of peripheral nerve as well as Schwann cell myelination. The role of the Hippo pathway in peripheral nerve regeneration has just started to be explored. After injury, Schwann cells adopt new identities to promote regeneration by converting to a repair-promoting phenotype. While the reprogramming of Schwann cells to repair cells has been well characterized, the maintenance of such repair phenotype cannot be sustained for a very long period, which limits nerve repair in human. First, we show that short or longterm myelin maintenance is not affected by defect in YAP and TAZ expression. Using crush nerve injury and conditional mutagenesis in mice, we also show that YAP and TAZ are regulators of repair Schwann cell proliferation and differentiation. We found that YAP and TAZ are required in repair Schwann cells for their redifferentiation into myelinating Schwann cell following crush injury.

Acknowledgement

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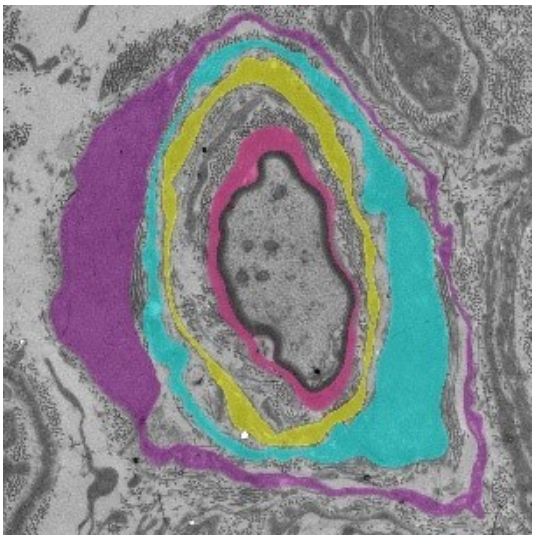
YAP and TAZ regulate Schwann cell proliferation and differentiation during peripheral nerve regeneration. Jeanette H, Marziali LN, Bhatia U, Hellman A, Herron J, Kopec AM, Feltri ML, Poitelon Y, Belin S. Glia. 2020 Dec 18. doi: 10.1002/glia.23949.



Formation of onion bulb in PNS remyelination

S27-04

Mechanisms controlling pns myelin maintenance

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Myelin, the multilamellar structure enwrapping the axons in Central (CNS) and Peripheral Nervous system (PNS) is crucial to ensure efficient propagation of the electric impulse, to execute complex nervous system functions and to maintain the integrity of the axons. Myelin is formed and maintained by continuous communication between axons and myelinating glial cells, Schwann cells (SC) in the PNS and oligodendrocytes in the CNS. This continuous communication is necessary for the correct development of the nervous system and to ensure a permanent and efficient transmission of the electric impulse. Recent studies have shown that this reciprocal communication is also key to regulate the metabolic coupling between axons and glial cells, as glial cells provide axons with essential metabolites to sustain their activity and functionality.

We previously reported that in PNS, Prostaglandin D2, one of the final products of the arachidonic acid metabolism, is synthesized by neuronal Prostaglandin D2 synthase (L-PGDS). Further, we demonstrated that loss of L-PGDS enzymatic activity causes hypomyelination at earlier stages of development and myelin aberrations and degeneration in adulthood.

We now show that in the absence of L-PGDS Schwann cells accumulate arachidonic acid, the substrate for prostanoid synthesis. In vivo lipidomic and metabolomic analyses indicate a complete derangement in the synthesis of lipids belonging to the omega-6 fatty acid family, which are involved in the arachidonic acid metabolism. These changes are accompanied by a significant decrease in the main metabolites of the Krebs cycle, thus suggesting altered glial cell metabolism. Notably, these variations are present only in aged mice and correlate with morphological myelin alterations. In vitro transcriptomic analyses confirmed that key enzymes involved in lipid

metabolism and synthesis are also altered when L-PGDS is not enzymatically active. Finally, in vitro metabolic flux analyses on myelinated Schwann cells neuronal cocultures demonstrate that in the absence of L-PGDS, Schwann cells undergo a metabolic rewiring to ensure glial cell and neuronal survival during aging.

Collectively we posit that L-PGDS is critical to maintain myelin integrity. Our data also indicate that Schwann cells are extremely plastic as they can adapt their metabolic demand to maintain a functionally active PNS.

S28 | Modulating reactive astrogliosis: a therapeutic strategy

S28-01

Parsing out the role of resting and reactive astrocytes in disease

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Astrocytes play integral roles in regulating the function of the nervous system. Their importance in the nervous system has been augmented in recent years by a multitude of studies that show that astrocytes play critical active and not merely passive roles in the brain. For instance, astrocytes have been shown to be important in regulating the later stages of myelination. Using a simplified *in vitro* neuronal-oligodendrocyte co-culture system that has been adapted for high-content imaging and analysis, we have identified a novel proteoglycan secreted by astrocytes that regulates myelination. We showed that this proteoglycan can also facilitate myelin repair after demyelination. To understand how reactive astrocytes contribute to disease states, we analysed the secretome and expression profiles of reactive and resting astrocytes. Strikingly, we found that reactive astrocytes produced lower levels of multiple proteins and that this had consequences on downstream processes. Overall, we highlight the different roles that astrocytes can play when resting and when reactive.

S28-02

Astrocyte microglia cross-talk in prion diseases and beyond

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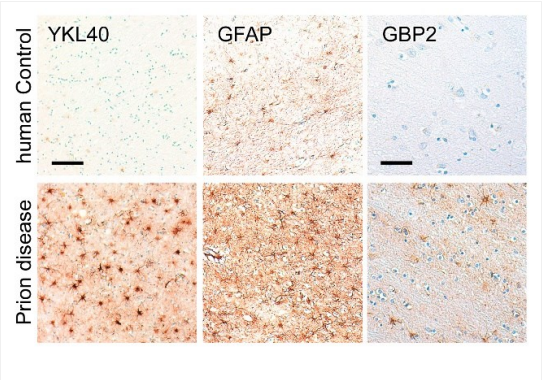
Prion diseases are fatal infectious neurodegenerative disorders affecting humans and animals alike. Prion diseases are the prototype of neurodegenerative diseases with protein misfolding, where the endogenous cellular prion protein (PrP^{C}) is converted into its misfolded isoform (PrP^{Sc}). PrP^{Sc} is highly resistant to degradation and accumulates in the brain leading to neurotoxicity. Beside deposition of misfolded PrP^{Sc} and neuronal loss, prion diseases are characterized by astrogliosis and the activation of microglia. The latter are highly activated in prion diseases and gradually lose their protective and homeostatic functions. This microglia phenotype switch is distinct from other neurodegenerative diseases and might be directly driven by PrP^{Sc}. Astrogliosis is common in neurodegenerative diseases, but highest in human prion disorders (see Figure) and murine prion models. However, the dysregulation of astrocytes in prion diseases is not well characterized. Both cell types, microglia and astrocytes, were viewed to act rather independently in disease pathophysiology, with proinflammatory microglia

considered to be the potential neurotoxic species at late disease stages. However, recent investigations showed that both cell types might be tightly linked: a proinflammatory microglial cytokine cocktail containing TNF a, IL 1a and C1qa might reprogram astrocytes to change their expression profile and phenotype, thus becoming neurotoxic (designated as A1 astrocytes). Thus, we wanted to assess if A1-like-astrocytes are abundant in prion diseases, how they might affect prion disease pathophysiology, and if their abolishment might be a therapeutic option for treatment. We could show that $C3^+$-reactive-astrocytes are highly abundant in prion disease mouse models and human prion diseases. We then investigated the impact of these astrocytes on prion disease pathophysiology by prion infecting TNF a, IL 1a and C1qa triple knockout mice, which are unable to develop A1-astrocytes. Generation of prion aggregates was unchanged, although the formation of $C3^+$ astrocytes was significantly reduced. However, general activation of astrocytes was very strong and showed a mixed phenotype that is distinct from other neurodegenerative diseases. Unexpectedly, triple cytokine KO led to a significant acceleration of prion disease course. This was accompanied by early dysregulation of microglial homeostatic markers. Taken together our data rather exclude the abolishment of $C3^+$-astrocytes as a therapeutic strategy in prion diseases.



Astrogliosis is highly abundant in human and murine prion diseases
The marker proteins for astrogliosis GFAP and YKL-40 are highly increased in human prion diseases. Moreover the A1 astrocyte protein GBP2 is highly abundant. However, their impact on disease pathophysiology is not well understood. Scale bars 100 and 50µm, respectively.

S28-04

Effects of modulating astrocyte reactivity via loss-of-function manipulations

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Astrocytes exhibit an evolutionarily ancient response to all forms of CNS injury and disease commonly referred to as astrocyte reactivity. Causation-testing transgenic manipulations and other technologies over the last twenty years have revealed that modulation of astrocyte reactivity can powerfully influence outcome in all types of CNS disorders. Nevertheless, understanding of the mechanisms and functions of astrocyte reactivity and how these may

differ in different disorders is only beginning. Studying the effects of loss-of-function manipulations that target the ablation of astrocytes or target the deletion of specific molecules selectively from astrocyes can powerfully advance the understanding of basic biology and provide insight regarding potential beneficial effects or detrimental consequences of manipulations being considered as therapeutic approaches. Here, we will examine and discuss the effects of a variety of different types of molecular and cellular loss-of-function manipulations on astrocyte reactivity and on the roles that reactive astrocytes play in various contexts including regulating inflammation, influencing axon regeneration and influencing tissue repair in a variety of disorder contexts including traumatic injury, autoimmune inflammation and degenerative disease.

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S29 | Extracellular vesicles shape glia-neuron interplay in health and disease of the nervous system

S29-01

Extracellular vesicles secreted by ethanol-treated astrocytes participates in glial-neuron communication spreading the neuroinflammation via TLR4

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Current reports demonstrate the role of extracellular vesicles (EVs) in the intercellular communication in physiological and pathological conditions, including neurodegenerative disorders. We have previously shown that ethanol activates glial cells through Toll-like receptor 4 (TLR4) by triggering neuroinflammation. Here, we evaluate if ethanol and the TLR4 response change the release and inflammatory content of astrocyte-derived EVs, and whether these vesicles are capable of communicating with neurons by spreading neuroinflammation. We have demonstrated that EVs derived from ethanol-treated astrocytes could be internalized by cortical neurons in culture, increasing several inflammatory compounds (e.g., proteins and miRNA levels) and compromising their survival. Ethanol is also able to increase the number of secreted nanovesicles and their content in inflammatory proteins (e.g. NFκB-p65, NLRP3, caspase-1, IL-1β) and miRNAs (e.g. mir-146a, mir-182) in the EVs from the WT-astrocytes compared with those from the untreated WT cells. No changes were observed in either the number of isolated EVs or their content between the untreated and ethanol-treated TLR4-KO astrocytes. Considering that EVs/exosomes can cross the blood brain barrier, the content of EVs in miRNAs, specific proteins (e.g. neuroglobin) and lipids can be used as potential biomarkers of the neuroinflammation-induced by alcohol. In summary, astrocyte-derived EVs could act as cellular transmitters by spreading the neuroinflammatory response induced by ethanol through TLR4 activation and contributing to the brain damage and neurodegeneration.

S29-02

Oligodendrocyte-derived exosomes in axon-glia interaction and glial support

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Myelinating oligodendrocytes provide support to axons, which is incompletely understood. The presentation will highlight the transfer of oligodendrocyte-derived extracellular vesicles (exosomes) to neurons and provide evidence that glia-to neuron exosome transfer is important for neuronal homeostasis and maintenance. I will demonstrate recent results obtained with CreERT2-reporter mice and conditional deletion of genes associated with exosome biogenesis to genetically interfere with exosome secretion, providing proof of glia to neuron exosome transfer in different brain regions. Furthermore, I will discuss the functional role of oligodendroglial exosomes in axonal maintenance and show that oligodendroglial exosomes derived from mice affected by secondary axonal degeneration (PLP- and CNP-ko mice) lack the ability to support the neuronal metabolism and to promote axonal transport. Together, our work demonstrates that glia to neuron exosome transfer is a mode of glial support important for long-term axonal maintenance and integrity.

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S29-03

Extracellular vesicles act as disease propagator in amyotrophic lateral sclerosis and frontotemporal dementia

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two uncurable neurodegenerative diseases that affect cognitive and motor areas of the central nervous system (CNS). In both ALS and FTD, motor and cortical neuron death first occurs in focal areas in the frontotemporal lobes or motor cortex and spinal cord, but eventually spreads across contiguous and connected regions of the whole central nervous systems (CNS), ultimately leading to widespread neurodegeneration and neuroinflammation, and death of the patients. ALS and FTD are believed to be two opposite ends of the same disease spectrum. The driving force and the biological mechanisms at the basis of the spreading of these diseases are still largely unknown but pivotal to bring ALS and FTD to a halt. An aberrant G_4C_2 repeats expansion in the intronic region of the *C9orf72* gene is the most common genetic cause of both ALS and FTD. A repeat-associated non-AUG translation (RAN-T) of the G_4C_2 expansion leads to the production of five different, aggregation-prone dipeptide repeat proteins (DPRs; poly-GA, poly-GP, poly-GR, poly-PA, poly-PR), that are linked to various toxic mechanisms inside cells. In order to preserve the proteostasis, cells try to remove harmful materials from their environment and one route is the production of

extracellular vesicles (EVs). EVs are released by neurons in an activity-dependent fashion and their composition is highly dependent on the intrinsic pathological status of the neuron or more in general the cell in which they are produced. Employing both in vitro and in vivo techniques we report that EVs produced by neurons are loaded with DPRs that are then incorporated into still healthy neurons triggering and boosting RAN-T. Interestingly, DPR^+ EVs are also incorporated into astrocytes and microglia which are part of the cellular milieu surrounding neurons. We observed that incorporation of DPR^+ EVs was able to cause *in vivo* astrogliosis and microgliosis, thereby accelerating the spread of disease mechanisms and neuroinflammatory processes. A deeper understanding of EVs involvement and role could open the possibility to find new therapeutic targets to halt the progression of ALS and FTD.

Acknowledgement

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S29-04

Dysfunctional astroglial exosome signaling to (motor) neuron axon in ALS models

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Amyotrophic lateral sclerosis (ALS) is a typical neurodegenerative disease in which both upper and lower motor neurons (UMNs and LMNs) undergo degeneration. Dysfunctions of MN axons that form both descending motor tracts and peripheral nerve tracts are widely observed in amyotrophic lateral sclerosis (ALS), which often precede MN soma (and axon) degeneration and significantly contributes to disease pathology. Although astroglia conditioned medium (ACM) derived from the mouse SOD1 mutant model or from human ALS patient brain astroglia is able to substantially modulate health and survival of MNs, astroglial factors in ACM that modulate MN survival in ALS conditions remain unidentified. In the current study, we investigated how astroglia-derived exosomes, a major type of secreted extracellular vesicles (EVs) that are originated from endosomes, affect (motor) neuron survival especially axon properties in ALS. By optimizing a filtration and size exclusion chromatography (SEC)-based purification of astroglial exosomes, we are able to minimize contamination of known astroglial proteins, such as Thrombospondins (TSPs), Hevin, Sema3a, and Sparc, with exosomes. Interestingly, astroglial exosomes from non-transgenic (NTg) control astroglia ACM are able to strongly stimulate neuronal axon growth and protect neurons from glutamate-induced excitotoxity. Expression of SOD1G93A mutant and cytokine treatment in astroglia significantly reduced astroglial exosomes' stimulatory and protective effect on neuronal axons. Unlike exosomes prepared from ultracentrifugation method, astroglial exosomes prepared from SEC method have no evident association with mutant SOD1G93A or other misfolded SOD1. We further found that NTg astroglial exosomes activates neuronal focal adhesion kinase (FAK) by increasing its tyrosine (Tyr) 397 phosphorylation. Pharmacological blockade of FAK also diminishes NTg exosomes' stimulatory effect on neuronal axons. In addition, proteomic analysis of NTg and SOD1G93A astroglial exosomes with cytokine treatment also found that HepaCAM, a glia-specific cell adhesion molecule (CAM), is abundantly and selectively expressed on NTg astroglial exosomes that is reduced by the expression of SOD1G93A and cytokine treatment. Selective silencing of HepaCAM is sufficient to significantly reduce NTg astroglial exosomes' stimulatory effect on neuronal axons. By employing our newly generated cell-type specific exosome reporter mice (CD63-$GFP^{f/f}$) and focal AAV-gfap-Cre

injections, we also found that astrocyte-derived exosomes travel significantly less in the spinal cord in SOD1G93A mice compared to littermate NTg mice. Overall, our current study suggests a loss-of-function mechanism of astroglial exosome HepaCAM signaling to (motor) neurons in ALS, adding new insights in understanding astroglia-mediated pathogenic mechanisms in ALS.

S30 | Exploring the regenerative properties of Müller glia for retinal repair

S30-01

Müller glial cell reprogramming and retina regeneration in zebrafish

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Although Müller glial cells (MG) in the zebrafish retina share structure and function with their mammalian counterparts, only in zebrafish are they able to function as retinal stem cells. In zebrafish, MG respond to neuron death by undergoing an asymmetric division that produces a proliferating population of multipotent retinal progenitors for retinal repair. Although factors regulating MG's decision to divide remain mostly unknown, a certain threshold of neuron death must be exceeded in order for MG to engage in a regenerative response. The reason why MG in zebrafish can function as retinal stem cells while those in mammals do not remains unknown. However, we anticipate that certain gene expression programs that are differentially expressed in zebrafish and mammalian MG will be involved. Indeed, one such program is that controlled by Notch signaling, which is restricted to MG in the developing and adult zebrafish retina while in mammals, Notch signaling is only transiently active in postnatal MG. In the adult zebrafish retina, we find that Notch signaling regulates MG's injury response threshold via its action on chromatin compaction and the expression of certain regeneration-associated genes. These mechanisms suggest new strategies for imparting an enhanced regenerative potential on mammalian MG.

Acknowledgement
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S30-02

Müller cells respond to focal laser injury with either retinal regeneration or reactive gliosis

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Retinal regeneration from Müller cells (MCs) is limited in mice compared to zebrafish (ZF), a species with high regeneration capacity. In the former, MCs undergo reactive gliosis that ultimately results in retinal damage. We have employed a model of focal laser-induced retinal degeneration in the outer nuclear layer to investigate the outcome in the two species. Subsequently, kinetics of MC activation, proliferation and regeneration respectively

gliosis was characterized. Thereby, we identified transforming growth factor β (TGFβ), which is essential for wound healing including scar formation and regeneration, as main player. Differential regulation of the TGFβ isoforms was observed in ZF and mouse MCs. Whereas TGFβ3 promotes retinal regeneration via Smad-dependent canonical signaling in the ZF, TGFβ1 and TGFβ2 evoke the p38 MAPK non-canonical pathway in the mouse, inducing reactive gliosis and thereby precluding retinal regeneration. Due to its pleiotropic nature, TGFβ may affect other physiological mechanisms. In this regard, our investigations into injury-induced changes revealed TGFβ/Notch interplay during reactive response of the MCs. Thereby, TGFβ1/2 and Notch1/2 interact to reprogram murine MCs towards an epithelial phenotype and ultimately to form a glial scar. Similar observation in gliotic MCs in drusen-positive human retina samples confirmed such epithelial appearance. The observed transition was pharmacologically modified in the mouse by the gamma secretase inhibitor DAPT, pirfenidone and the selective inhibitor of Smad 3 (SIS3). Complementary, arresting the cell cycle in ZF MCs by palbociclib changed a mesenchymal, regenerative phenotype towards a gliotic response due to Tgfβ1 and Notch1/2 expression. In conclusion, modulation of the MC stemness by reversing an epithelial transition during injury response may open new avenues into remodeling regeneration in degenerative retinal diseases.

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S30-03

Interplay between Müller glia and microglia during retinal regeneration

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While teleost or amphibian Müller glial cells efficiently sustain retinal regeneration, their mammalian counterparts are unable to do so. *Xenopus* is a particularly valuable model to explore mechanisms that either sustain or constrain the regenerative potential of Müller glia. Indeed, we unexpectedly discovered that Müller glia dependent retinal regeneration is hampered in young *Xenopus* tadpoles, while being efficient in old premetamorphic ones. We found a remarkable correlation between these different Müller cell capacities and the status of microglia, the resident immune cells of the retina. We will present our data suggesting that the differential responses of Müller glia cells to injury in *Xenopus* may originate from differences in the inflammatory reaction. This should contribute to a better understanding of the coupling between inflammation and regeneration.

S30-04

Insights into the inhibitory controls that prevent murine Müller glia regeneration in the retina

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Müller glia are endogenous glial cells in the retina that act as stem cells in fish and amphibians, replacing retinal cells lost due to injury. In contrast, mammalian Müller glia have lost this ability to self-heal and instead activate a negative cytotoxic process known as reactive gliosis, that serves to limit the spread of injury. In fish and amphibians, where Müller glia have a proliferative response, the first step is the delamination and interkinetic nuclear migration of glial nuclei, followed by cell cycle re-entry to become progenitor/stem cell like. While mammalian Müller glia undergo these initial events in response to injury, they rapidly abort the regenerative process and no meaningful cell replacement is achieved. We have gained new insights into the inhibitory events that prevent a fulsome Müller glia regenerative response, focusing on two tumour suppressor genes. Firstly, we have found that*Phosphatase and tensin homolog (Pten)* plays a role in regulating the delamination of Müller glia. Secondly, we have found hat Plagl1, which is maternally imprinted and encodes a zinc finger transcription factor, prevents Müller glia proliferation. The identification and characterization of these latent regulators will better position us to design gene therapies that could activate Müller glia for regenerative purposes in the clinic.

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Workshops

W01 | Imaging white matter function and structure

W01-01

Imaging functional interactions between axons and oligodendrocytes *in vivo*

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The complex process of myelination requires the intimate interaction of axons and oligodendrocytes. It is now clear, for instance, that oligodendrocyte-lineage cells can be highly responsive to the activity of associated axons, but the underlying cellular and molecular mechanisms remain incompletely understood. Remarkably, these responses can occur rapidly following neuronal activity changes (minutes to hours), as shown in a variety of approaches from in vivo mouse models to MRI studies of white matter microstructure. These fast responses indicate that we would benefit from being able to assess the functional state of these cells at high temporal resolution to study how neuronal activity can influence myelination. This is difficult in an intact nervous system that preserves all axon-oligodendrocyte interactions. The zebrafish embryo model, however, offers an excellent platform to address these questions: their small size, transparency, external fast development and transgenic and genetic accessibility enable their intact CNS to be imaged repeatedly at unprecedented resolution.

Here, we describe how we are using genetically encoded functional reporters to study axon-myelin interactions in zebrafish. We employ SypHy, a reporter of synaptic vesicle release, to determine how activity-dependent neurotransmitter exocytosis from axons regulates their myelination, and identify axonal hotspots of glutamate release that can promote myelin elongation in vivo early in development. We also employ myelin-tethered calcium reporters (GCaMP7) to assess the functional state of oligodendrocytes and myelin sheaths at high spatio-temporal resolution in vivo. In agreement with axonal SypHy results, we find that calcium dynamics play an important role early on, in newly-differentiated oligodendrocytes, where they can predict sheath growth or retraction, but then decrease with animal development; and we find that there is some contribution of neuronal activity to overall myelin calcium activity. We will also discuss how we can use zebrafish to simultaneously probe axonal and myelin functional states, and to directly image glutamate sensing in oligodendrocytes by using novel glutamate biosensors

(SFiGluSnFR). We will highlight the potential of these approaches to dissect the underlying molecular mechanisms of axon-myelin interactions, to test the importance of intracellular calcium dynamics in regulating myelination, and how it can be expanded to illuminate myelinated axon biology, for instance during demyelination and regeneration.

W01-02

Optic nerve imaging to study axon-glial functions

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Cellular mechanisms of white matter glial support for axonal function are poorly understood and difficult to investigate. Genetically-encoded optical sensors allow assessing cellular ion and metabolite dynamics in real-time and are invaluable tools to study the interplay between neurons and glial cells. We use a combination of optic nerve electrophysiology with two-photon biosensor imaging to investigate the impact of glial physiology on myelinated axons and vice versa. Biosensor expression in glial cells or neurons/axons can be achieved by transgenic approaches or by viral mediated strategies in mice. We show the potential of the optic nerve as a model system to interrogate cellular mechanisms of axon-glial interactions and the regulation of axonal energy homeostasis. With the plethora of biosensors and the use of mouse lines (conditional knockouts or disease models) optic nerve imaging allows to further understand axon-glia functions in physiology, pathology and aging. This imaging technique is a powerful and versatile approach, opening exciting avenues for future studies on the molecular and cellular mechanisms governing axon-glia functions.

W01-03

The many ways to image myelin with the electron microscope

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Since the early days of electron microscopy this imaging techniques has been instrumental in studying myelin ultrastructure. With the sophisticated sample preparation and 3D imaging methods in combination with elaborated mouse models manifold opportunities are available nowadays to learn more about white matter.
In electron microscopy sample preparation deserves special attention. In the past cryopreparation techniques like high-pressure freezing and freeze substitution proved useful for preservation of myelin if fine structural details are studied. For other questions also preparation by chemical fixation is useful, for example in the case of localization by immunoelectron microscopy or determination of myelin thickness. Imaging large areas by scanning electron microscopy or the application of volume imaging techniques such as focused ion beam scanning electron

microscopy (FIB-SEM) require other preparation steps because of the different imaging modality. Here we will illustrate which protocols are suitable to address specific questions by providing examples of our research in the field of myelin turnover, myelin protein and lipid functions.

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W01-04

Glia on Tape –3D Ultrastructure and Targeted Electron Microscopy

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For a detailed understanding of myelin morphology 3D EM assessment has become increasingly instructive. One technique combines the collection of ultrathin tissue sections on tape with serial scanning EM and volume reconstruction. Here we show that this method can be applied to gain a spatial understanding of myelin wrapping exemplified by double myelination in a mouse model devoid of adhesion complexes. This technique is ideally suited to screen for certain morphological features or specific cellular events in a given 3D tissue context. We used this to investigate cell body myelination in a zebrafish larvae model which is a rare event that can only be fully characterized by its ultrastructure. We exploited volume EM to relocate a single oligodendrocyte after light microscopy (LM). A cortical oligodendrocyte was laser-ablated and the remyelination pattern assessed by longitudinal two-photon microscopy. For correlated LM-EM (CLEM) near infrared branding (NIRB) was used to create as landmarks to find the myelinated axons by EM. We found that preexisting, reestablished and de novo myelin sheaths formed by a novel oligodendrocyte were morphologically similar.

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W02 | Physiological functions of adult microglia: implications for plasticity, learning and memory

W02-01

Microglial calcium signaling is attuned to neuronal activity in the awake mouse

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Microglial calcium signaling is an often-dormant component of microglial physiology during in vivo studies of the basal state (1,2,3). However, a number of conditions can evoke greater microglial calcium activity, including instances of pathology (1,2) and neuronal activity changes (3). We describe microglial calcium signaling in the awake mouse cortex using 2-photon microscopy, with an emphasis on exploring microdomain signaling across a number of activity states. While only a subset of microglial processes and somata exhibit spontaneous activity, elevating local neuronal activity using Gq DREADD (AAV-CaMKIIa-hM3D-mCherry) increases microglial process, but not somatic, calcium activity in an agonist-dependent manner. Similarly, the AMPA and Kainate receptor agonist, kainic acid, can provoke greater microglial process calcium signaling during the acute seizures of status epilepticus. In addition the neuropathology that accompanies kainic acid administration results in sustained microglial calcium elevations over a 10 day period, involving prolonged, whole-cell calcium events and spreading calcium waves. On the other hand, reducing local neuronal activity using Gi DREADD (AAV-CaMKIIa-hM4D-mCherry) can also evoke an unexpected elevation in microglial process, but not somatic, calcium signaling. Application of isoflurane to induce general anesthesia also results in profound elevations in microglial process calcium activity. Across these different approaches, microglial process calcium elevations were not immediate, and instead developed approximately 10-15 minutes after peak neuronal activity changes. In addition, the processes most associated with calcium elevations underwent structural extension. Overall, our work suggests microglial calcium elevations may be an important component of the dynamic structural responses of microglia as they sense changes in local or widespread neuronal activity shifts.

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W02-02

Investigation of Microglial Morphodynamics during Vigilance States by Two-Photon imaging

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Microglial cells, the resident immune cells of the brain, have numerous and highly dynamic processes in physiological conditions. Various studies suggested that beyond a possible role in surveillance of the parenchyma, microglial dynamics could be linked to synaptic mechanisms and neuronal activity. A better understanding of the purpose and functions of microglial morphodynamics in physiological conditions is thus an important question to tackle. Thus, there is an urgent need for non-invasive approaches allowing to image microglia and to monitor neuronal activity in vivo. One of the great challenges of studying “resting” microglial morphodynamics in vivo lies in the fact that these cells are functionally designed to detect subtle changes of their immediate environment and to react by a rapid change in morphology. In extreme cases, this leads to a quite static and poorly ramified morphology that is associated to neuroinflammation.

This presentation will focus on the methodologies that we developed to image microglial cells, as well as the concurrent image processing. In particular, we performed imaging in non-anesthetized mice through the thinned skull while monitoring electroencephalogram and electromyogram. This combination of technics and analysis allowed us to quantify changes in motility and process ramifications over the shift of wake and sleep. These experiments provide further details on the regulation of microglial morphodynamics by neuronal activity in physiological conditions and raise interesting questions on the role of microglia during vigilance states.

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W02-03

Studying the microglia cytoskeleton: impact of Rho GTPase ablation in microglia morphology and function

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Microglia, the largest immune resident cell population in the brain, constitute around 10% of all the glial cells in the adult brain. Within the brain parenchyma, microglia are responsible for immune surveillance, innate immune responses, and the sculpting of the neuronal circuitry. These cells constantly extend and retract their cell processes to survey the brain parenchyma and suffer profound changes when triggered. Morphological changes are likely to require a very dynamic reorganization of the microglial cytoskeleton. Thus, understanding how the cytoskeleton reorganizes itself and how key molecules regulating actin dynamics can impact microglia will give us new perspectives on how these cells execute their functions. Rho GTPases are not only key players in actin cytoskeleton dynamics but can also regulate gene expression and signaling transduction. RhoA, Rac1 and Cdc42 functions have been extensively studied in cells from the CNS, but little attention has been paid to microglia. Our lab is focused in understanding both the roles of Rho GTPases in glial cells and the impact of microglia dysfunction in other CNS populations. Using conditional gene ablation, we have ablated RhoA, Rac1 and Cdc42 specifically in adult microglia to understand the function of these proteins in the physiology of microglia at organism level. Concomitantly, we also use Förster resonance energy transfer (FRET) in live microglia cell cultures to explore the signaling pathways and subcellular functions regulated by RhoGTPases. Our results show that RhoA ablation in microglia led to morphological changes, acquisition of a pro-inflammatory phenotype with production of inflammatory mediators, and increase in glutamate release, which resulted in neurotoxicity and development of an Alzheimer's Disease-like phenotype reproducing several hallmarks of the disease, including cognitive deficits. On the other hand, Rac1 ablation in microglia generated a completely distinct phenotype. Loss of Rac1 in adult microglia did not result in a pro-inflammatory phenotype and the morphological changes were not as pronounced as the ones observed in microglia without RhoA. In addition, microglia without Rac1 became irresponsive to pro-inflammatory stimuli, such as LPS, failing to expand, and having reduced production of ROS and lower levels of NF-κB activation. Interestingly, while there were no alterations in the number of neurons, there was a significant reduction in the number of excitatory synapses in the hippocampus and the neocortex, accompanied with behavioral changes. Overall, our observations suggest that Rho GTPases are essential for microglia and CNS homeostasis maintenance, reinforcing the central importance of the actin cytoskeleton for microglia cell biology.

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W02-04

Defective neuron-microglia crosstalk: impact on functional maturation of hippocampal synapses

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Microglial cells are the resident immune cells of the central nervous system. Emerging roles of microglia are related to their ability in continuously scanning the brain environment, maintaining tissue homeostasis and participating in network formation. During postnatal life, through direct interaction with neurons, microglia actively participate in synapse remodeling events. Nevertheless, the mechanism of such interactions and their impact on synaptic function remain obscure.
Using two different models, we studied how microglia shape neuronal networks in hippocampus. Firstly, disruption of the neuron-microglia CX3CL1/CX3CR1 axis strongly impairs developmental maturation of excitatory hippocampal synapses, leading to abnormal functional features of the presynaptic terminal. More recently, adopting a pharmacological depletion strategy, we highlighted the role of microglia in supporting glutamatergic synapses in hippocampus, contributing constitutively to maintain neuronal functions throughout the life.

W02-05

Microglial phagocytosis modulates adult hippocampal neurogenesis

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Adult neurogenesis is limited to specific brain regions such as the hippocampus, which is involved in memory- and depression-related behaviors. This process is regulated by a wide range of molecular and cellular factors, and among them stand out microglia. Microglia, the innate immune cells of the brain, regulate neurogenesis through several functions, including the phagocytosis of apoptotic newborn cells. But, how does microglial phagocytosis of apoptotic cells modulate the production and survival of newborn neurons? We have recently shown that mice deficient in phagocytosis receptors MerTK and P2Y12 show a reduction in adult neurogenesis. In addition, we found that the intrahippocampal administration of phagocytic microglia secretome reduces the proliferation and survival of newborn neurons in the hippocampus. However, these approaches do not allow us to modulate specifically microglial phagocytosis nor generate greater changes on adult neurogenesis, as the effects of the secretome were localized to the injection site. Therefore, we are now using a pharmacological approach to inhibit

microglial phagocytosis and assess its downstream effects on adult neurogenesis and neurogenesis-related behaviors. To find novel compounds that affected either engulfment or degradation of apoptotic cells, we performed a high throughput screening in primary microglial cultures of 600 compounds of the Prestwick Library, already approved by the Federal Drug Administration (FDA) and the European Medicines Agency (EMA) to be used in humans. We found five drugs, whose targets are neurotransmitter receptors, which inhibit phagocytosis in cell cultures. To validate these drugs as phagocytosis inhibitors in a more complex system, we used organotypic cultures. We are currently testing these drugs in vivo to block microglial phagocytosis and study the effects on adult neurogenesis and behavior. All the approaches and findings mentioned before suggest that phagocytic microglia modulate the homeostasis of adult hippocampal neurogenesis.

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W02-06

Behavioral outcomes of microglial dysfunction across the life span

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Over the last twenty years, remarkable findings have transformed our understanding of how microglia contribute to brain functioning and homeostasis. First, microglia-neuron interactions are critical for synaptic maturation and refinement of neuronal circuits during postnatal development. Accordingly, manipulation of a number of microglial genes during postnatal development leads to long-lasting behavioral defects, suggesting that primary deficit in microglia contribute to circuit-level deficits typically found in neurodevelopmental disorders. Moreover, in addition to their well-established impact on brain maturation, recent studies unveiled a major role for microglia during adulthood, modulating neuronal activity and shaping synaptic and structural plasticity, thus affecting learning, memory and forgetting. However, their role in supporting cognitive processes in the healthy adult brain is only beginning to be understood.

In my presentation, I will first discuss the use of behavioral tasks to study microglial functions across the life span. Certain behavioral domains, such as stereotypic behavior, sociability and flexibility, seem to be affected when primary microglial functions are disrupted during development, but not later; while other behaviors, such as learning and memory, rely on adult microglial functioning. I will then focus on our recent discoveries which, based on behavioral approaches, identified serotonin (5-HT) as an upstream modulator of microglial functions both during development and throughout adulthood. These findings suggest that, during early postnatal development, 5-HT signaling in microglia is required to modulate synaptic refinement, underlying proper sociability and flexibility in adulthood. Moreover, throughout life, 5-HT signaling in microglia modulates learning-induced spine remodeling and memory consolidation.

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W03 | Thyroid hormone action in glial biology

W03-01

Modeling Thyroid Hormone Action in human glia by using Induced Pluripotent Stem Cell

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Thyroid hormone is essential for normal human brain development. Untreated congenital hypothyroidism or disorders due to defects in Thyroid hormone signaling result in severe neurodevelopmental problems. Human models to investigate Thyroid hormone regulation in neural cells are lacking and animal models have limitation mimicking the human physiology. We employ induced Pluripotent Stem Cells (iPSCs) technology as an advanced unique human model to understand general Thyroid hormone physiology in neural cells both in health and disease. We aim to understand the basic properties of Thyroid hormone regulation in different glial cells during early brain development, and how presence or absence of Thyroid hormone affects glial biology. We generate different neural lineages mimicking early neurodevelopmental stages from wild type iPSCs; Neurons, astrocytes, Neural Precursor Cells and Oligodendrocyte Precursor Cells by using cell type-specific-differentiation protocols. We perform Thyroid hormone transport and metabolism assays using radiolabeled Thyroid hormone to understand the regulation of Thyroid hormone in glial cell in health and disease. We could show the baseline characteristics of Thyroid hormone transport and metabolism in different neural lineages. Our results indicate that each neural cell lineage has unique features of Thyroid hormone signaling. Utilizing neural cell lineages differentiated from human iPSCs provide a unique tool to model Thyroid hormone physiology and disorders associated with disrupted Thyroid hormone signaling.

W03-02

The role of transthyretin in oligodendrocyte development and central nervous system myelination in rodents.

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Thyroid hormones are important to all vertebrates, especially in crucial brain developmental processes, such as myelination, a process where axons of nerve cells are insulated to protect the axon and nerve from damage and to

provide it with an essential electro-physical property which facilitates accurate coordination of information to and from the brain. The absence or damage to myelin from axons in the central nervous system can lead to the loss of brain function which can present as symptoms associated with acquired demyelinating diseases, such as multiple sclerosis or dysmyelination in various inherited congenital diseases. Myelination in the central nervous system is dependent on considerable numbers of oligodendrocytes to produce myelin in normal development and to restore myelin in disease states. Transthyretin (TTR) is a thyroid hormone distributor protein which facilitates movement of thyroid hormones in the periphery and more specifically across the blood-cerebral spinal fluid barrier and into cerebral spinal fluid. Interestingly, a hypermyelination phenotype was described in the corpus callosum of TTR null mice, suggesting a possible role for TTR in regulating developmental myelination. However, the extent of the effect that TTR has on oligodendrocyte production in mature adult mice, with direct relevance to acquired disease, is still yet to be fully elucidated. To cultivate and consolidate the body of knowledge pertaining to the role of TTR in the adult oligodendroglial precursor cell response to neural injury and repair, wild type and TTR null mice have been used in experiments that set out to: 1. Determine whether the deletion of TTR affects remyelination following cuprizone-induced demyelination of the adult mouse corpus callosum; 2. Identify signalling pathways critical for TTR-dependent myelination; 3. Determine if TTR effects the regulation of oligodendrocyte maturation in the corpus callosum, sub-ventricular zone and in the rostral-migratory axis; and 3. Establish whether TTR regulates adaptive immune cells in tissue and in circulation.

W03-03

Hypothalamic tanycyte-neuron interaction evokes global changes in thyroid hormone economy

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Thyroid hormone (TH) signalling plays a critical role in the development and maintenance of the central nervous system. TH economy is centrally regulated by the hypothalamo-pituitary-thyroid (HPT) neuroendocrine axis, that is primarily responsible to maintain relatively stable circulating TH levels. However, TH signalling is also regulated on the local level by a complex intracellular machinery that involves TH metabolism as a critical component. By operating this local regulatory system in a cell-type specific manner, tissue specific regulation of TH action (THA) is achieved. A key characteristic of tissue-specificity is the selective expression of deiodinases, the main players in TH metabolism. Deiodinases activate and inactivate TH; type 2 deiodinase (D2) is the main activator converting T4 to the active TH form, T3, while type 3 deiodinase (D3) is responsible for TH inactivation. These two processes are compartmentalized in the brain. TH is activated in the D2 expressing glial compartment and provide T3 for neurons which only express D3 thus are unable to activate TH. This compartmentalization is especially important in the hypothalamus, where D2 expressing specialized glial cells, called tanycytes are responsible for TH activation and presenting T3 to hypophysiotropic TRH neurons of the paraventricular nucleus (PVN), representing a crucial hypothalamic regulator of the HPT axis. Thus, the local regulation of THA in the hypothalamus and pituitary represents a functional interconnection between the local regulatory circuit and the HPT axis with impact on the control of global TH economy.Non-thyroidal illness syndrome (NTIS) is a common endocrine condition represented by the lacking or inadequately low central response of the HPT axis to falling circulating TH levels. It is accompanied with increased hypothalamic D2 activity but up to know no direct evidence could be obtained on local, brain region specific THA on under this condition. We subjected our recently generated Thyroid Hormone

Action Indicator (THAI) transgenic mouse to LPS-induced NTIS and directly measured local THA and other related parameters in this metabolic challenge. Our data indicate that tanycyte-evoked increase in D2 activity results in localized increase of THA in the mediobasal hypothalamus that consequently downregulates TRH expression in hypophysiotropic TRH neurones of the PVN and this neuro-glial interaction results in global downregulation of TH economy. Importantly however, these changes evoke specific responses in various organs, and while some organs moderate their local THA, some are unaffected or even in an enhanced state. Thus, in NTIS, eu, hypo and hyperthyroid conditions co-exist at the same time in the same animal. It is currently under study, how aging, a potent challenger of TH economy, can impact this process.

Acknowledgement

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W03-04

A multidisciplinary approach exploring the role of the thyroid hormone transporter MCT8 in oligodendrocyte maturation and myelination.

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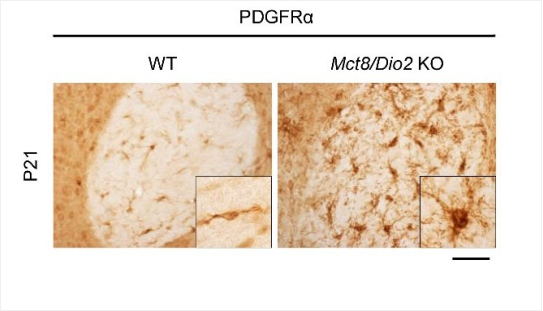
Oligodendrocytes are glial cells that play a crucial role in the CNS, where they produce the myelin sheaths that insulate axonal processes of neurons enabling saltatory conduction of action potentials and providing metabolic axonal support. Oligodendrocyte maturation and myelination are finely regulated processes that require key trophic signals important for growth and metabolism. Thyroid hormone (TH) is a potent signal that regulates several processes such as oligodendrocyte maturation, myelination, and oligodendroglial synaptic interactions. TH transport across the blood-brain barrier and cellular membranes is mediated by a specific transmembrane transporter, the monocarboxylate transporter 8 (MCT8). Dysfunction of MCT8 results in impaired TH uptake in the developing brain that leads to inherited hypomyelination and psychomotor disabilities in the X-linked Allan-Herndon-Dudley syndrome (AHDS) or MCT8-deficiency. Even though the altered myelin status in AHDS patients is one of the main hallmarks of the disease, it is still a matter of debate whether there is a permanent hypomyelination or a delay on myelination that is restored later in life.

To address this point, we have made use of multiple approaches to study myelination processes in *Mct8/Dio2* knockout mice (KO), an already validated model for AHDS, from postnatal to adult stages, to gain new insight into the pathophysiological mechanisms of AHDS and the effects of TH on myelination.

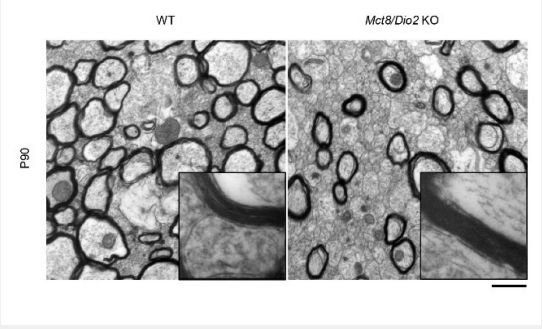
Myelination was studied, first of all, histologically by assessing the content of both myelin proteins and lipids. These studies showed persistent myelination defects in the brain of *Mct8/Dio2* KO mice and were also in agreement with observations at the ultrastructural level showing severely decreased percentage of myelinated axons in the *Mct8/Dio2* KO mice brain using transmission electron microscopy analyses. Myelination was also assessed by means of the state-of-the-art MRI-based neuroimaging technique, Diffusion Tensor Imaging (DTI), which is used to estimate the myelin organization and content in the brain, revealing brain microstructural alterations in parameters used to evaluate myelinating disorders which, together with the histological data, corroborates that *Mct8/Dio2* KO mice replicate the myelination impairments reported in the patients and shows that these alterations are still

present at adult stages. Data obtained on myelination led to the study on oligodendroglial dynamics, showing altered proliferation and differentiation patterns from oligodendrocyte precursor cell stages.

Myelination in *Mct8/Dio2* KO mice is altered from early developmental stages, when also oligodendroglial dynamics suffer several impairments, and these alterations persist throughout later stages with only partial recovery. Altogether, these data provide new understanding on the effects of TH on myelination, and on the pathophysiological mechanisms underlying MCT8-deficiency to design and evaluate possible future treatments.



Altered proliferation patterns from oligodendrocyte precursor cells in the Mct8/Dio2 KO mice brain.
Altered oligodendroglial development in *Mct8/Dio2* KO mice. Brain coronal sections of P21 WT (n = 4) and *Mct8/Dio2* KO (n = 4) mice were immunostained with anti-PDGFRα. Representative images depict the immunostaining pattern in the anterior commissure. PDGFRα immunopositive cell density is increased in the *Mct8/Dio2* KO mice. Insets depict PDGFRα positive cells in both WT and *Mct8/Dio2* KO mice. *Mct8/Dio2* KO mice OPCs show an aberrant increase in size and branching as compared to WT.



Severely decreased percentage of myelinated axons in the Mct8/Dio2 KO mice brain.
Altered myelination pattern in *Mct8/Dio2* KO mice. Ultrathin sections of the medial part of the cc were analyzed by electron microscopy at P90 (n = 6). The number of myelinated axons was severely decreased in *Mct8/Dio2* KO mice as compared to WT animals. No differences in the ultrastructure of the myelin sheaths were observed between both genotypes as seen in the higher-magnification insets.

W03-05

Developmental thyroid hormone action determines the later-life neuro/glia output in the murine subventricular zone

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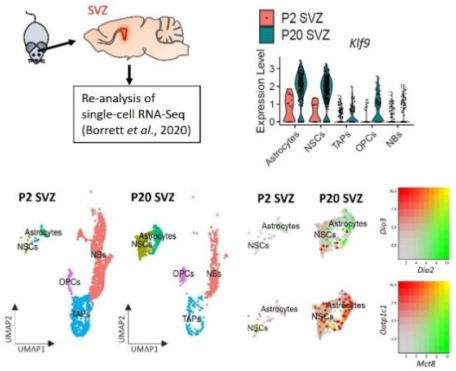
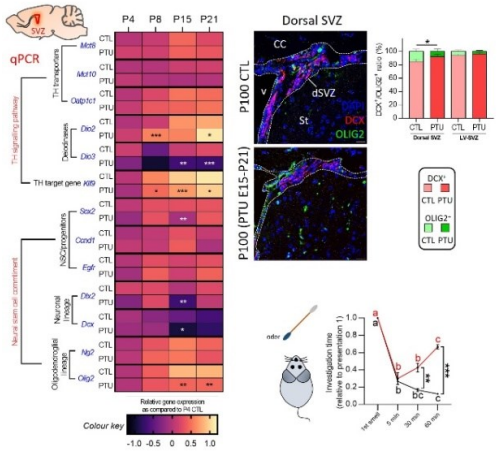
The subventricular zone (SVZ) of the adult mammalian brain harbors neural stem cells (NSCs) that generate neurons and oligodendrocytes throughout life. Single-cell RNA-Seq analysis on mouse SVZ-NSCs isolated throughout development showed they acquire their adult neurogliogenic identity between postnatal day (P) 7 and 20, establishing a stable neuro/glia output thereafter. However, factors governing this transition remain unknown. Driving transcriptional responses during brain development, we hypothesized that thyroid hormone (TH), whose levels rise postnatally and peak at P15, fulfils a role. Furthermore, TH directs SVZ-NSC fate choice in adults as well, with high intracellular TH favoring neuronal lineage commitment.

Re-analysis of the SVZ single-cell data at P2 and P20 revealed a dynamically increased expression of the TH transporters *Mct8* and *Oatp1c1*, and TH-(in)activating deiodinases *Dio2* and *Dio3* in NSCs, signs that local TH action is promoted (**figure 1**). Immunostainings showed a concomitant burst in SVZ-neurogenesis at P21. Expression of transthyretin (TTR), known as a TH-distributor in the cerebrospinal fluid, also peaked in P7 SVZ-cells. RNAscope showed several transcripts in most cells. This suggests an intracellular role for TTR too, to be tested on SVZ-NSCs *in vitro*.

Then, to study what occurs if TH synthesis is blocked, we fed dams a 0.15% propylthiouracil-enriched diet from embryonic day 15 to P21. Several TH target genes and those implicated in NSC commitment were dysregulated (**figure 2**). Postnatal hypothyroidism decreased cell mitosis at P4 and P21, but increased numbers of SOX2-positive SVZ-neuroprogenitors. In the dorsal SVZ, the main site of neurogliogenesis, less DCX-positive neuroblasts were detected at P21, while OLIG2-positive oligodendroglia numbers did not change. Next, we prepared *in vitro* neurospheres from dissected SVZs of control and PTU-treated mice, and allowed them differentiate with or without added T_3. The neuro/glia balance in cultures prepared from P4 animals of either condition did not change when T_3 was added, suggesting perinatal NSCs are irresponsive to TH. The balance did change in T_3-treated neurospheres prepared from control P21 animals, however, not in those from PTU-exposed mice, suggesting NSCs remain T_3-irresponsive in absence of the TH peak. Lastly, we examined 3-month-old mice that returned to a normal diet after developmental PTU exposure. Fewer oligodendroglia accounted for a permanently altered neuro/glia output in the dorsal SVZ (**figure 2**). Mice displayed a reduced ability to remember odors, indicating impaired olfaction, otherwise reliant on proper SVZ-neurogenesis. In addition, the olfactory bulbs were less enriched with the neuronal subtypes processing olfactory stimuli.Our data indicate that developmental TH signaling disruption permanently affects the SVZ neuro/glia output. These new read-outs allow to identify adverse outcome events on brain development and will permit comparison with events following exposure to endocrine disruptors.



Increased TH action during mouse SVZ development
Re-analysis of a single-cell RNA-Seq on the postnatal murine SVZ showed a dynamically increased expression of regulators of thyroid hormone signalling, including the TH transporters *Mct8* and *Oatp1c1*, the TH-(in)activating deiodinases *Dio2* and *Dio3*, and the TH-target gene *Klf9*. These are signs that local TH action in neural stem cells is promoted during postnatal SVZ development, when the later-life neuro/glia balance is established.



Blocked TH synthesis dysregulates gene expression and permanently alters the SVZ neuro/glia balance
Postnatal hypothyroidism alters the expression of genes in the SVZ that are implicated in TH signalling and neuroglial commitment. The developmental exposure to PTU that blocked TH synthesis, results in a permanently altered neuro/glia balance in the dorsal SVZ more then 2 months after the insult. As a consequence, adult mice showed a reduced ability to remember odors in a short-term memory test.