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INTRODUCTORY COURSE ON GLIAL BIOLOGY

I-01

Astrocytes - an introduction -

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By and large, the human brain consists of approximately 160 billion cells that mainly represent two types, classified as neurons and glia, each contributing about 50% to the total cell number. The cells of the brain are organized in functional and morphological networks and centers. Recent research has shown that glial cells may also act as neural stem cells, and are crucially involved in virtually every aspect of nervous system function, from early embryonic development to neurodegeneration and regeneration.

Astrocytes are probably the most versatile class of glia. Strategically positioned between the *pia mater* and the blood vessels of the brain as well as the neuronal synapses, astrocytes display a plethora of properties. They take up nutrients from the blood, metabolize them and provide energy substrates to neighbouring neurons. They contribute to the blood-brain barrier, link neuronal activity to changes in the blood flow, promote synapse formation, and determine the properties of the extracellular matrix. Equipped with transporters and ion channels they regulate ion and transmitter levels in the extracellular space thereby regulating synaptic transmission. An exciting recent finding was that astrocytes secrete low-molecular-weight compounds such as ATP, glutamate or D-serine, thereby actively modulating neurotransmission. This impressive list demonstrates on how many routes astrocytes interact with neurons and influence brain activity.

To investigate the role of astrocytes a variety of genetically modified mice have been generated that facilitate numerous methodological approaches. Targeting fluorescent proteins to astrocytes allows the analysis of morphological changes, migration or proliferation during development or injury situations. Genetically encoded indicator proteins have been used to monitor physiological signals, e.g. Ca^{2+} transients. But recent developments will highlight the dynamics of metabolic ATP changes or redox status. Most frequent are astrocyte-specific knockout animal models that link molecular function to animal behavior.

I-02

Oligodendrocytes

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50% of the human brain is comprised of myelinated axons - so-called white matter. These axons are wrapped by multilamellar myelin sheaths formed by oligodendrocytes, cells whose function has long been thought of simply to generate these sheaths that act as passive insulators to promote rapid impulse conduction. However it is clear from recent studies that I will introduce and discuss that oligodendrocytes also play central roles in the metabolic support of the axon – a function that provides key new insights into the causes of neurodegeneration in the myelin disease multiple sclerosis. Furthermore, with our increased understanding of how the myelin sheath is generated and the evidence for plasticity of the sheaths in the adult CNS, I will address the question as to whether myelin plasticity, alongside neuronal plasticity, might contribute to the most fundamental property of the brain - the ability to learn.

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I-03

Microglia: a reintroduction

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Microglia arise during primitive hematopoiesis and initially enter the developing murine brain around E10.5, before other glia and prior to neuronal differentiation. During development, microglia interact extensively with neurons, helping to establish neuronal populations through influences on survival, apoptosis and corpse-clearance. As neuron-to-neuron contacts are forming during early postnatal life, microglia refine neuronal network properties by synaptic pruning.

Neuron-microglial and microglial-neuron dialogs are mediated by contact-dependent and soluble signals. As one example, fractalkine (CX3CL1) is a transmembrane chemokine expressed in the CNS solely by neurons, which also release soluble fractalkine to signal at a distance. The fractalkine receptor (CX3CR1) is restricted to microglia among CNS cells. Perturbation of fractalkine/fractalkine receptor signaling results in altered microglial function, with consequences observed from mid-gestation through aging and neurodegeneration. Using mice deficient for fractalkine or its receptor provides a wealth of insights into microglial function in the intact CNS and may have direct clinical relevance, given a common hypomorphic CX3CR1 allele in humans. Examination of the mechanism underlying fractalkine regulation of microglial responses may yield information useful for translating model results to clinical application.

I-04

Schwann cells

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In peripheral nerves, Schwann cells exist in two forms: myelin and non-myelin (Remak) Schwann cells. Remak Schwann cells accommodate small diameter axons along troughs on their surface, whereas myelin Schwann cells elaborate thick insulating myelin sheets around large diameter axons. Mature Schwann cells found in adult nerves originate from the neural crest in well-defined developmental steps: first neural crest cells differentiate into Schwann cell precursors, which then generate immature Schwann cells, which in turn, give rise to myelinating and non-myelinating Schwann cells. These cells are essential for nerve development and repair, and for nerve function, most notably, in elaborating myelin sheaths for rapid saltatory conduction of nerve impulses.

The myelination process is an exquisite process relying on profound gene expression, morphological and structural changes amongst others. This is controlled by axon-associated signals, including neuregulin-1 and BACE 1, and other extra-cellular signals that include BDNF, GDNF and IGF1/2. On a molecular level, this process is regulated by several key transcription factors that include Egr2, Sox-10 and Oct6 amongst others, and epigenetic mechanisms, including DNA methylation and histone modifications.

A highly unusual feature of Schwann cells is that they are highly plastic cells that can undergo a radical change in phenotype in response to different insults. This is most strikingly seen in injured nerves, where loss of axonal contact transforms the myelin and Remak Schwann cells to “repair” Bungner Schwann cells, in a process largely controlled by the Notch and ERK signaling pathways, and the transcription factor c-Jun. This remarkable plasticity of Schwann cells has also been notably demonstrated after microbial infections, whereby the leprosy bacterium has been shown to reprogram adult Schwann cells to a stage of progenitor/stem-like cells. Whereas this adaptive response of Schwann cells is beneficial after nerve injury, since it is central to the striking regenerative potential of the peripheral nervous system, in the case of microbial infections, it is detrimental since it promotes bacterial spread and dissemination of infection.

I-05

Gliogenesis

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Much attention has been attracted by the capability of a neural stem cell population to generate new neurons in the adult brain. However, in parallel and in a similar fashion to what has been observed during brain development, adult hippocampal NSCs generate astrocytes in a process linked to their activation and their depletion, with a direct impact in aging and neurological disorders such as epilepsy. We will explore the biological mechanisms by which NSCs generate astrocytes in health and disease and will subject to scrutiny the pros and cons of the different novel technological approaches employed to study NSCs, neurogenesis and gliogenesis.

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I-06

Advances and challenges in studying the role of astrocytes in neurotransmission

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Accumulating evidence provided by numerous laboratories during the past decades have demonstrated an active role of astrocytes in regulating synaptic transmission in different brain areas.

I will present the current evidence supporting the involvement of astrocytes in synaptic function, discussing the properties of the astrocytic responsiveness to synaptic activity through activation of neurotransmitter receptors that lead to intracellular calcium elevations in astrocytes. I will also present the experimental results that have identified the consequences of these calcium elevations in the control of synaptic transmission and plasticity through the release of gliotransmitters that activate neuronal receptors.

I will finally discuss the experimental challenges that need to be overcome and the novel potential tools that may be useful to help to better understand the actual role of astrocytes in sensing and regulating synaptic function in situ and in vivo.

For that purpose, I will emphasize the importance of achieve a high control of cellular specificity to selectively stimulate astrocytes, and the relevance of the temporal and spatial control of such

stimulation, and the importance of mimicking physiological stimuli to decipher the actual meaning of astrocyte activity on synaptic physiology and brain function.

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I-07

Experimental models of myelination and remyelination

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The development of *in vitro* and *in vivo* models raised significant progress in the characterization of the cellular and molecular mechanisms regulating myelination and remyelination in the central nervous system. These experimental models have been instrumental for addressing the pathogenesis of myelin disorders such as multiple sclerosis and leucodystrophies. The most commonly used models to study the pathophysiology of these myelin diseases are the experimental encephalomyelitis (EAE), the viral-induced demyelinating models and the toxin-induced models of demyelination. In this glial biology course, I will review and discuss recent advances on the preclinical models of myelination/remyelination, their limitations, their pitfalls and how they can be used to develop rational therapeutics for myelin diseases, such as multiple sclerosis.

I-08

Glia in Alzheimer´s disease

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Alzheimer disease (AD) is the most common form of dementia and the most prevalent neurodegenerative disease in the elderly population. AD is characterized by a familial early onset form which has a genetic component related to mutations of three genes (presenilin-1, presenilin-2, and amyloid b protein precursor), and a sporadic, late-onset form (95% of the cases) that is mainly associated with aging. AD progression has been associated with a gradual damage in function and structure in the hippocampus and neocortex, the vulnerable brain areas used for memory and cognition. The disease is recognized as a multifactorial disorder with combined defects: Ab plaques, tautopathy, neurofibrillary tangles, neuronal loss as well as, glial reactivity and neuroinflammation are associated with the disease.

Glial cells are responsible for homoeostasis of the nervous tissue by executing a wide array of housekeeping functions. When the brain is exposed to adverse conditions, the glial cells acquire specialized 'reactive' or 'activated' phenotypes that relate to the characteristics of the insult. Gliosis is associated with essential beneficial functions, but under specific circumstances can lead to harmful effects. In AD, clusters of activated microglia and reactive astrocytes have been observed near amyloid beta deposits. Potential dysfunctions of these cells and gliosis are being identified that can contribute to, or be primary causes of AD.

AD has not been widely considered a disease of white matter (WM), but more recent evidence suggests the existence of abnormalities in myelination patterns and myelin attrition in AD-afflicted human brains. WM tracts within the cerebral cortex and subcortical regions, which are comprised of myelinated white fibers, provide connectivity that is central to cognition, emotion, and consciousness. Myelination occurs in a "heterochronological" pattern. Studies of affected areas in the AD brain, including the hippocampus and entorhinal cortex, suggest that the regions most vulnerable to AD pathogenesis exhibit the most protracted course of myelination. Cells comprising the oligodendocyte lineage mediate the process of myelination, and AD related insults may detrimentally impact the ability of these cells to properly myelinate axonal processes in vulnerable brain regions. This lecture will illustrate the main dysfunctional properties of glial cells in AD. Recent work on the new role of glia in the AD progression will be discussed.

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I-09

Microglia in the universe of myeloid cells in the CNS

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The diseased brain hosts a heterogeneous population of myeloid cells, including parenchymal microglia, perivascular cells, meningeal macrophages and blood-borne monocytes. To date, the different types of brain myeloid cells have been discriminated solely on the basis of their localization, morphology and surface epitope expression. However, recent data suggest that resident microglia may be functionally distinct from bone marrow- or blood-derived phagocytes, which invade the CNS under pathological conditions. During the last few years, research on brain myeloid cells has been markedly changed by the advent of new tools in imaging, genetics and immunology. These methodologies have yielded unexpected results, which challenge the traditional view of brain macrophages. On the basis of these new studies brain myeloid subtypes can be differentiated with regard to their origin, function and fate in the brain.

WORKSHOPS

Workshop 01
Beyond cell culture: modern techniques to study microglia

W01-01

Preparation of chimeric OHSCs to study the function of ramified microglia

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Microglia are the resident CNS immune cells constituting the first-line of defense in brain-injury. They are characterized by a typical ramified morphology in the healthy brain, while in the diseased brain microglia show a more macrophage-like morphology having shortened and thickened processes and sometimes amoeboid shape. During the last decade it has been established that microglia are by no means inactive under so-called resting conditions instead they are highly active and constantly surveil their environment. However, uncovering the function of ramified microglia has just begun. The lack of knowledge about these cells under normal conditions is mostly due to the fact that microglia are experimentally very difficult to approach. They are very reactive cells that rapidly respond to any brain disease or injury; thus, culturing them inevitably causes activation and to functionally investigate ramified microglia *ex vivo*, *in situ* or *in vitro* was so far was very difficult, if not impossible.

Organotypic hippocampal slice cultures (OHSCs) are a well-accepted tool to study different neurobiological aspects including neuron-glia interactions. This culture model combines a very close *in vivo* situation with relatively easy possibilities of experimental manipulation to investigate numerous neurobiological questions. We present here a method to deplete and replenish ramified microglia in OHSCs. Microglia are depleted with the macrophage toxin clodronate. For replenishment adult microglia are added on top of the tissue. These cells invade the depleted OHSC throughout the hippocampal layers, acquire a regular distribution and regain a ramified morphology. This protocol enables researchers to investigate the function of ramified microglia in a complex cell culture setting that mimics numerous *in vivo* properties. The presented protocol is also suitable for the generation of chimeric OHSCs. For this purpose microglia from a different genotype than the host OHSC are used for replenishment. Thereby it is possible to examine the effects of genetic modifications (knockouts or overexpression of transgens) specifically in microglia or the rest of the tissue. This straight forward approach is saving time and is reducing costs, effort and animal numbers compared to the laborious generation of microglia-specific genetically modified mice.

W01-02

Acute isolation of microglia and bioinformatics

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Acute isolation of pure microglia populations from mouse, rat, human and other species provides the possibility to isolate high quality RNA, DNA and protein samples. Indeed an increasing number of genome-wide transcriptome profiles from pure populations of microglia is coming available and allows for systematic study of glia cells in health and disease. Different types of bioinformatic analyses can be applied to these transcriptome datasets. Differential gene expression analysis is used to identify genes that are significantly up or down-regulated with a selected fold change between two conditions of interest. More recently, gene expression analysis of transcriptome datasets generated with RNA sequencing has yielded quantitative gene expression data. Co-expression analysis has been applied to generste gene expression networks for specific microglia functions. Impressive studies on microglia-based functionality using transcriptome and epigenetic analysis have been published recently (1-5). In this presentation we will review the most recent advancements in microglia isolation and gene expression profiling.

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W01-03

Studying the role of dicer and microRNAs in microglia of the developing and adult brain

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As the resident macrophage population of the central nervous system (CNS), microglia arise during early development from embryonic yolk sac-derived CX_3CR1^+ progenitors and then persist throughout the life of the organism with limited self-renewal. Microglia act as immune sentinels coping with infection or tissue damage. Moreover, recent studies suggest that they are also involved in the establishment and maintenance of neuronal circuits, regulating neurogenesis, neuronal differentiation and synaptic remodeling. To protect the CNS from collateral damage, microglia activity is thought to be tightly regulated by various factors, including microRNAs (miRs). Here, we report animals carrying conditional miR deficiencies in microglia, generated by crossing *Cx3cr1*^{Cre} or *Cx3cr1*^{CreER} mice with animals, harboring a conditional allele encoding dicer, the main miR-processing enzyme. In *Cx3cr1*^{Cre}*:dicer*$^{fl/fl}$ animals, microglia are targeted during embryonic development, while *Cx3cr1*^{CreER}*:dicer*$^{fl/-}$ microglia can be targeted postnatally, upon tamoxifen administration. Strikingly, prenatal impairment of microglial dicer expression resulted in pronounced microglia hyper-activation, followed by a delayed motor neuron deficiency and hind limb defect in the adult mice. In contrast, the postnatal introduction of the dicer deficiency in newborn or adult microglia was compatible with brain homeostasis and mice, unless challenged, did not display any overt phenotype. Interestingly though, and in line with earlier reports of the involvement of dicer and non-coding RNAs in the repair of DNA double-strand breaks, the *dicer* deficiency turned radio-resistant adult WT microglia radiosensitive. Collectively, our results highlight the importance of miRs in microglia-dependent establishment and maintenance of neuronal networks during embryonic development, but suggest that adult microglial homeostasis is uniquely robust in that it can be maintained in absence of post- transcriptional control by miRs.

W01-04

In vivo imaging of microglia in the brain and spinal cord in health and disease

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In the adult brain, microglia were long believed to stay in a dormant/resting state, activated only in the event of an insult to the brain. This view changed dramatically when it became possible to image microglial behavior in the intact brain over time; in vivo imaging thus revealed the dynamic nature of microglial responses and brought increasing interest towards the role of microglia in numerous physiological and pathological paradigms. In vivo imaging studies of microglia became possible due to three major technological advances: 1. The generation of microglia reporter mice; 2. The development of two-photon microscopy; 3. The development of imaging methods that allowed optical access to the central nervous system (CNS) for longitudinal imaging studies in living animals. This presentation will provide a brief introduction to two-photon microscopy and will discuss available imaging methods used to study microglia in the brain and spinal cord of living mice, highlighting how in vivo imaging has reshaped our understanding of microglial biology in the healthy and diseased CNS.

W01-05

Imaging neuron microglia interaction in the hippocampus of awake mice

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In vivo imaging represents a powerful tool to study microglia in their natural environment, which led to the discovery of their fine-process motility. Moreover, imaging of microglia in relation to blood vessels and neurons revealed important physiological and pathological functions.

Up to now, microglia have been analyzed in superficial cortical areas of anesthetized mice. Despite the importance of the hippocampus for learning and memory, knowledge about microglia motility and interaction with neurons is scarce for this brain region. Moreover, the influence of anesthesia on microglia motility remains unknown.

Here we present a novel technological approach to perform in vivo imaging of microglia and neurons in the hippocampus. This preparation will enable the recording of microglia motility in awake mice during learning and memory processes.

W01-06

Improvement of classical methods and development of high throughput automated methods for the analysis of glial morphology

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Understanding changes in glial morphology is now a widely used tool to gain insight into how these cells respond to different environmental and genetic challenges. Surprisingly, despite the many recent advances that have been made in microscopy, such as multiphoton and super-resolution imaging, there have been comparatively few developments in how the resulting images are analysed. This situation recently motivated our research team to closely examine classically use image analysis tools, beginning with the most common technique, image thresholding. Our survey of this technique revealed that conventional thresholding of images is in most cases unlikely to yield clear or relevant information about the nature of differences between groups. In response we have developed a new set of procedures that should add significantly to both the clarity and interpretation of results using this technique. Additionally, we have recently begun to develop higher throughput automated reconstruction algorithms for glia that allow us to circumvent the extremely tedious processes involved in manually reconstructing these cells. These new algorithms allow the reconstruction of a vastly

greater number of cells in a fraction of the time, a situation that significantly adds to the overall representativeness of morphological reconstruction data sets. These issues and others relevant to the morphological assessment of glia will be discussed.

WORKSHOP 02
Manipulation and visualization of physiological and pathophysiological functions of glia

W02-01

Neurons and glia on the edge of cortical spreading depression

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Cortical spreading depression is a slowly propagating wave of near-complete depolarization of brain cells followed by temporary suppression of neuronal activity. Accumulating evidence indicates that cortical spreading depression u*nderlies the migraine aura and that similar* waves promote tissue damage in stroke, trauma, and hemorrhage. Cortical spreading depression is characterized by neuronal swelling, profound elevation of extracellular potassium and glutamate, multiphasic blood flow changes, and drop in tissue oxygen tension. The slow speed of the cortical spreading depression wave implies that it is mediated by diffusion of a chemical substance, yet the identity of this substance and the pathway it follows are unknown. Intercellular spread between gap junction-coupled neurons or glial cells and interstitial diffusion of K^+ or glutamate have been proposed. Here we use extracellular direct current potential recordings, K^+-sensitive microelectrodes, and two-photon imaging with ultrasensitive Ca^{2+} and glutamate fluorescent probes to elucidate the spatiotemporal dynamics of ionic shifts associated with the propagation of cortical spreading depression in the visual cortex of adult living mice. Our data argue against intercellular spread of Ca^{2+} carrying the cortical spreading depression wavefront and are in favor of interstitial K^+ diffusion, rather than glutamate diffusion, as the leading event in cortical spreading depression.

W02-02

Understanding calcium-driven physiology of astroglia

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Astrocytes appear to rely on relatively sluggish and spatially blurred calclium waves to communicate with fast and point-precise neural circuits. This apparent discrepancy could however reflect our current inability to understand the microscopic mechanisms involved in generation and propagation of calcium-dependent physiological signals across glial cells. Difficulties in detecting and interpreting such signals may have led to some prominent controversies in the field. Here we will focus on two principal factors that appear to be hampering our progress here: the extraordinary nanoscopic morphology of astroglia and the woes of interpreting astrocytic calcium signal recordings. We will discuss some emerging methodologies that might accelerate our quest to understand glia, such as time-resolved fluorescence imaging and advanced biophysical models of realistic astroglia. We will argue that progress in this area requires a qualitative leap in our experimental and analytical strategies.

W02-03

Imaging of astrocyte sodium dynamics in (patho-)physiology

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Sodium ions are important charge carriers for electrical brain activity. In addition, they provide the driving force for various transport processes across the plasma membrane. Among these, glutamate removal from the extracellular space by astrocytes is of outstanding relevance for proper neural function. Notably, maintenance of the sodium gradient by the Na^+/K^+ATPase constitutes one of the most energy consuming processes in the brain and many studies indicate that sodium dysbalance is among the first events provoked by metabolic stress.

Here, we will present sodium imaging as a reliable tool to monitor intracellular sodium concentration under physiological as well as pathophysiological conditions in acute tissue slice preparations of the mouse brain. With two-photon excitation of the fluorescent sodium indicator SBFI, measurements of sodium dynamics in fine astrocyte processes can be achieved. Moreover, we present quantitative data on sodium transients in single cells as well as multicellular networks obtained by ratiometric widefield imaging.

Using the latter approach, we found that hippocampal astrocytes *in situ* rarely show spontaneous sodium changes. Application of transmitters/agonists as well as synaptic transmitter release, however, resulted in clearly detectable sodium increases due to the activation of glutamate uptake. Depending on the strength of stimulation, these transients can be limited to single processes, occur within the entire cell, or even spread into neighbouring, coupled cells. During pharmacologically-induced epileptiform activity, network wide sodium oscillations developed in both neurons and astrocytes. Discharge duration and neuronal sodium loads were restricted by astrocyte glutamate removal which relies on sufficient glial energy production. Limited glucose availability or impaired glial metabolism increased sodium baseline in astrocytes, attenuated glial glutamate removal and strongly affected neuronal sodium signals. Additionally, neuronal signal kinetics were affected by glucose deprivation or inhibition of glycolysis. Lactate as an alternative metabolic substrate could rescue the effects on neuronal but not on astrocyte sodium signals. These results show that astrocytes limit electrical discharges and sodium load of neurons during network sodium oscillations. Furthermore, they indicate that astrocytes provide metabolic support to neurons thereby ensuring neuronal sodium balance, a task that can be compromised under pathological conditions.

W02-04

How does an oligodendrocyte select axons to myelinate?

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Oligodendrocytes myelinate multiple axons in the central nervous system (CNS). Conduction velocity of myelinated axons is at least 50 times faster than that of unmyelinated axons. Myelin is also important for axonal survival, because oligodendrocytes metabolically support axons via myelin. In contrast to a Schwann cell that myelinates axons in the peripheral nervous system, one oligodendrocyte myelinates multiple axons in the CNS. Recent paper reports depolarized oligodendrocytes increase conduction velocity of neuronal axons they myelinate. This brings up an idea that depolarization of one oligodendrocyte increase conduction velocity of multiple axons that are myelinated by one oligodendrocyte simultaneously.

To address this issue, it is important to understand the interaction between each oligodendrocyte and neuronal axons more precisely. High density of oligodendrocytes in the CNS prevents us from observing each oligodendrocyte morphology by the regular method to visualize oligodendrocytes. We found attenuated rabies virus (RV) sparsely labels oligodendrocytes in the white matter and adeno-associated virus type2 (AAV2) efficiently labels neuronal axons. Interaction between each

oligodendrocyte and neuronal axons was successfully detected by dual injection of attenuated RV and AAV2 into the brain.

We revealed that there are 3 groups of callosal oligodendrocytes. There were significant differences in the mode of myelination between callosal and chiasmal oligodendrocytes. We also showed that activity dependent myelination does not occur after initiation of myelination. These finding suggested that neuronal activity affects initiation of myelination but neuronal subtypes are not selected by each oligodendrocyte after the initiation of myelination.

W02-05

In vivo imaging of glial Ca^{2+} dynamics using an ultrasensitive Ca^{2+} indicator

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Astrocytes generate local Ca^{2+} signals that are thought to regulate their functions. To visualize these signals in intact brain, we generated transgenic mice expressing the ultrasensitive ratiometric Ca^{2+} indicator yellow Cameleon-Nano 50 (YC-Nano50) in astrocytes. We first generated a knock-in mouse line in which the tet operator (tetO)-YC-Nano50 cassette was inserted downstream of the b-actin gene locus. We then crossed these mice with *Mlc1*-tTA mice in which the expression of tetracycline transactivator (tTA) is driven by an astrocyte-specific promoter. Using these mice, we detected a previously unidentified pattern of Ca^{2+} signals, which occur spontaneously, predominantly in astrocytic fine processes but not the cell body. Upon strong sensory stimulation, astrocytes initially responded with Ca^{2+} signals at fine processes, which then propagated to the cell body. These observations suggest that astrocytic fine processes function as a high-sensitivity detector of neuronal activities. Thus, the method provides a useful tool to study the activity of astrocytes in brain physiology and pathology. In addition, through the selection of other tTA mouse lines, subcellular Ca^{2+} signals may be studied in other glial cell types such as oligodendrocytes and microglia as well as in neurons and vascular smooth muscle cells. Using such mice relationships among the spatiotemporal patterns of Ca^{2+} signals in neurons, smooth muscle cells and astrocytes are now being studied *in vivo*.

W02-06

KENGE-tet system: promising strategy to achieve sufficient probe expression in glial cells

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To dissect neuron-glia interaction, especially to examine glia-initiated actions toward neurons, glial cell specific manipulation is required. Tetracycline-controlled gene induction system, called tet-system, can achieve cell-type specific gene expression of interest. However, the conventional tet-system merely gained the sufficient expression of functional probe in glial cells. We established Knockin-mediated ENhanced Gene Expression by improved tetracycline-controlled gene induction (KENGE-tet) and succeeded in sufficient expression of opsins in vivo. This method requires two lines of mice - one that controls the pattern of expression and another that determines the protein to be produced. Generation of new lines of either type readily expands the repertoire of targeting cell types and expressing probes.

PLENARY LECTURES

P-01

Fractalkinomics: one key to the place of microglia in the CNS firmament

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Microglia arise during primitive hematopoiesis and enter the developing murine brain around E10.5, before other glia and prior to neuronal differentiation. During development, microglia interact extensively with neurons, helping to establish neuronal populations through influences on survival, apoptosis and corpse-clearance. As neuron-to-neuron contacts are forming during early postnatal life, microglia refine neuronal network properties by synaptic pruning.

Neuron-microglial and microglial-neuron dialogs are mediated by contact-dependent and soluble signals. Fractalkine (CX3CL1) is a transmembrane chemokine expressed in the CNS solely by neurons, which also release soluble fractalkine to signal at a distance. The fractalkine receptor (CX3CR1) is restricted to microglia among CNS cells. Perturbation of fractalkine/fractalkine receptor signaling results in altered microglial function, with consequences observed from mid-gestation through aging and neurodegeneration. Using mice deficient for fractalkine or its receptor provides a wealth of insights into microglial function in the intact CNS and may have direct clinical relevance, given a common hypomorphic CX3CR1 allele in humans. Examination of the mechanism underlying fractalkine regulation of microglial responses may yield information useful for translating model results to clinical application.

P-02

Contribution of astrocytes to synaptic transmission and plasticity

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A growing body of evidence accumulated over the last decade has strongly suggested that cerebral communication was not just a neuronal matter but also involved glial cells, and in particular astrocytes, that enwrapped synapses and neurons. These results led to the emerging concept of the tripartite synapse that considers astrocytes as active partners of chemical synapses. It was established that astrocytes could detect synaptic activity through the presence on their membrane of receptors and transporters and respond in return through the release of signalling molecules that have been termed gliotransmitters by analogy to neurotransmitters. The ability of astrocytes to ensure neurotransmitter uptake and to release gliotransmitters and their impact on synaptic transmission and synaptic plasticity has raised a lot of attention, identifying astroglial cells as possible targets to generate new and effective therapeutic strategies for brain diseases. We have shown that astrocytes were contributing to synaptic plasticity through the release of D-serine, an endogenou co-agonist of NMDA receptors. More recently, we have provided evidence that membrane trafficking of the glutamate transporter GLT-1 in astrocytes was shaping the excitatory signal by facilitating the removal of glutamate from the synaptic cleft. It thus appears that astrocytes can contribute in a very significant manner to cerebral communication.

P-03

Intrinsic and extrinsic regulation of CNS myelination

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The signals regulating the spectacular cell-cell interaction by which oligodendrocytes generate myelin sheaths in the CNS remain poorly understood. Experiments from my lab and others show that axo-glial adhesion molecules alter the tempo and extent of CNS myelination, but are not required for the

process. These and other experiments to test the hypothesis that axonal signals are required have therefore, unlike the situation in PNS, failed to reveal any such signals necessary for CNS myelination. Experiments using inert microfibres suggest an alternative hypothesis - that myelination by oligodendrocytes results from intrinsic signalling pathways that can generate sheaths of physiological length without any molecular cues required from the axon. One of these intrinsic signaling pathways is that establishing polarity, as the basal polarity protein Scribble is required for normal CNS myelination and remyelination. Rather than being required for myelination, those extrinsic signals we have identified modify the number and length of sheaths. Together, this combination of intrinsic and extrinsic signalling may provide an adaptable developmental mechanism by which a hardwired pattern of myelination can be established to allow conduction, and then modified by external signals to enable plasticity.

P-04

Glial glycogen: a sweet and sour story full of surprises

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Glycogen is a brilliantly designed glucose storage molecule. While we understand quite precisely how glycogen functions in liver and muscle, this remains an emerging story in the nervous system. In fact, the very existence of glycogen in normal peripheral nerve was only recently established. What we have learned offers tantalizing insights about glial-neuronal metabolic interactions in both the central and peripheral nervous systems (CNS, PNS). We have studied the physiology and functions of glycogen in CNS white matter and peripheral nerve, using acutely isolated mouse optic nerve (MON), a typical CNS white matter tract, and mouse sciatic nerve (MSN). These advantageous preparations allow rigorous experimental assessment with quantitative electrophysiological techniques, including an 'enzyme' electrode for detecting lactate, biochemical glycogen assay and electron microscopic visualization of cytoplasmic glycogen particles. Glycogen is present in astrocytes in MON and in myelinating (but not non-myelinating) Schwann cells in sciatic nerve. Glycogen concentration is highest in MSN by a factor of two and depends on extracellular glucose concentration. Aglycemia causes loss of the stimulus evoked compound action potential (CAP) in both tissues after a latency that ranges from ~15 min in the MON to ~120 min in MSN myelinated axons (i.e., A fibers). CAP decline coincides with exhaustion of usable tissue glycogen, except in the case of unmyelinated sciatic nerve axons (i.e., C fibers) that are surrounded by Schwann cells that don't appear to contain glycogen. Increasing glycogen content greatly prolongs the latency to decline onset and, conversely, decreasing glycogen shortens this latency. The metabolic support provided by glycogen during aglycemia is abolished by an inhibitor of glycogen breakdown (DAB) in both MON and MSN. Both tissues exhibit high levels of extracellular lactate ($[Lactate^-]_o$), up to 50% of which derives from glycogen. During aglycemia, glycogen in astrocytes and Schwann cells is metabolized to lactate and 'shuttled' to axons to support oxidative energy metabolism. Glycogen breakdown and lactate transport to axons is also needed to sustain brief periods of intense axonal discharge. In summary, glial glycogen is a 'backup' source of energy substrate in both the CNS and PNS during glucose deprivation and subsidizes the energy demands of axons during times of intense activity when substrate demand outstrips supply.

P-05

Cell biology of myelin assembly and disassembly

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Myelin is formed by the spiral wrapping of oligodendrocyte plasma membrane around axons of the central nervous system. One important goal is to understand how oligodendrocytes form myelin, how they select axons for myelination and how they regulate myelin thickness/intermodal length. We will present a model of how CNS myelin elongates along and wraps around axons to form a tightly compacted multilayered membrane structure. This model may not only explain how myelin is generated during brain development, but could also help to understand myelin remodeling in adult life,

which may serve as a form of plasticity for the fine tuning of neuronal networks. Myelin disorders are among the most prevalent and disabling diseases in young adults. Although poorly understood, a consistent feature of myelin in various white matter diseases is the intralamellar vacuolization and fragmentation of the membrane. We will present a model of how myelin may loose its stability in such diseases.

P-06

Immune mechanisms of synapse loss in health and disease

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One of the major unsolved mysteries in neuroscience is how synapses are eliminated in the healthy and diseased brain. During development, neural circuitry undergoes a remodeling process in which excess synapses are eliminated and the remaining synapses are strengthened. This pruning process is required for precise brain wiring; however the mechanisms that drive the elimination of specific synapses in the brain remain unclear. Emerging evidence implicate molecules traditionally associated with the adaptive and innate immune system. For example, recent work from our laboratory revealed a key role for microglia and molecules traditionally associated the classical complement cascade in developmental synaptic pruning. Our recent studies support a model in which 'weaker' or less active synapses in the developing brain are targeted by complement proteins (C1q, C3) and then eliminated by phagocytic microglia that express receptors for complement and other immune molecules. These findings raise the question of how microglia differentiate the synapses or axons to prune from those to leave intact. Microglia-mediated synaptic refinement appears to depend on a careful balance of "eat me" (ie. complement) and a group of novel immune- related protective signals.

An early hallmark of many neurodegenerative diseases (NDDs) is a progressive, region-specific degeneration of synapses; however, molecular mechanisms that drive synapse loss remains elusive. Our recent work suggest that aberrant activation of some of these normal immune -related pruning pathways mediate early synapse loss in neurodegenerative diseases (NDDs), including Alzheimer's Disease (AD) and Huntington's disease (HD). Understanding how these immune mechanisms drive developmental pruning may provide novel insight into how to protect synapses in NDDS and other disorders of synaptic dysfunction, including autism and schizophrenia.

P-07

The night life of the brain

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We have recently described a macroscopic pathway in the central nervous system - the glymphatic system that facilitates the clearance of interstitial waste products from neuronal metabolism. Glymphatic clearance of macromolecules is driven by cerebrospinal fluid (CSF) that flows in along para-arterial spaces and through the brain parenchyma via support from astroglial aquaporin-4 water channels. The glymphatic circulation constitutes a complete anatomical pathway; para-arterial CSF exchanges with the interstitial fluid, solutes collect along para-venous spaces, then drain into the vessels of the lymphatic system for ultimate excretion from the kidney or degradation in the liver. The glymphatic system is only active during sleep. As such, this circulation represents a novel and unexplored pathway for understanding the biological necessity for sleep.

SYMPOSIA

SYMPOSIUM 01
Microglia-mediated control of postnatal brain development

S01-01

Development of microglia in the mouse primary somatosensory cortex

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Microglial cells are the resident macrophages of the central nervous system and play crucial roles in responses of the brain to various types of insults and pathologies, including different neurodegenerative diseases. In pathological conditions, microglia functions are underpinned by major changes in phenotype, named microglia activation, characterized by a number of structural, biochemical and functional changes, and influenced by ON and OFF signals emitted by neurons to instruct microglia on their status. During the normal development, increasing evidence indicates that microglia colonization of the central nervous system is related to neuronal development. Here we examined a role of microglia in the cortical development. We focused on the rodent thalamo-cortical system as a model system of neuronal circuit development and we combined the use of transgenic animals, immunohistochemistry and electrophysiology. Our data indicate that neuronal network development partly dictates the recruitment of microglia in the barrel field of the somatosensory cortex and suggest that microglial cells contribute to the maturation of cortical synapses.

S01-02

The induction of hippocampal long-term potentiation increases the motility of microglial processes and impacts their engagement with dendritic spines

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Mounting evidence indicates that microglia play important roles for normal brain physiology in addition to their actions as macrophages during brain pathology. Microglia have been implicated in the establishment of synaptic connectivity during brain development and its remodeling during experience-dependent plasticity. However, very little is known about how microglia dynamically interact with synapses, and whether these interactions are affected by the induction of synaptic plasticity.

We addressed these basic questions by two-photon imaging of microglia and CA1 pyramidal neurons together with electrophysiological recordings in acute mouse hippocampal brain slices. We analyzed the motility of microglial processes and the dynamics of their contacts with dendritic spines during basal synaptic transmission and after the induction of long-term potentiation (LTP).

We observed that at any given moment in time only a low fraction of spines formed contacts with microglial processes during basal synaptic transmission. However, the fraction increased substantially over time due to the highly dynamic nature of the microglial processes, allowing them to form multiple contacts in close succession. The induction of LTP increased the overall motility of microglial processes, while it reduced the number, but increased the duration of their contacts with dendritic spines. These effects were absent when the expression of LTP was blocked by the NMDAR antagonist APV.

Altogether, our study is consistent with the idea that microglia are actively involved in synaptic plasticity in the healthy adult brain, coming into contact with a substantial number of spines and being able to react to the induction of LTP, which causes them to engage more intimately with a subset of dendritic spines.

I will also briefly review our recent efforts to use super-resolution STED microscopy to investigate the dynamic interactions between microglial processes and dendritic spines at the nanoscale in acute brain slices.

S01-03

A role for microglia in synaptic plasticity

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The remodeling of circuitry is a fundamental aspect of brain function, contributing both to the initial wiring of the brain during development and to critical processes such as learning and memory in the adult. Changes to neural network function rely on structural and functional changes at synapses, and mechanisms which modulate synaptic changes critically contribute to cognitive function throughout the life span. Recently, microglia have come into the spotlight as regulators of synapses. These immune cells have been shown to display dynamic interactions with synapses in the non-pathological brain, although their contribution to synaptic plasticity is still poorly understood. Our work has characterized microglial contributions to experience-dependent plasticity in the visual cortex *in vivo*. We show that manipulations of visual experience elicit a remarkably rapid behavioral response in microglia which is distinct from their inflammatory response. This behavioral response includes an increase in phagocytosis that corresponds to the early phase of plasticity in this model when synapses are lost and when microglia increase their synaptic interactions. To determine the underlying mechanism behind this response, we examined the role of the chemokine fractalkine and the purinergic receptor P2Y12. Fractalkine signaling and purinergic signaling are two signaling pathways well studied for their neuroinflammatory roles, which are particularly well positioned to also function in the physiological process of synaptic rearrangement. Both pathways allow for specificity of signaling between neurons and microglia, and are capable of initiating chemotaxis of microglial cells. We report that unlike other systems where fractalkine is necessary for circuit remodeling, it is not required to achieve normal plasticity in the visual system. Conversely, we found that the purinergic receptor P2Y12 is critical for this late form of developmental plasticity. 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.

S01-04

Imaging microglia and synapses with an optical clearing technique

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Microglia play an important role in synapse formation and elimination. Direct contacts between microglial processes and synapses are thought to be a key event that regulates synapse stability. These contact events were shown to be transient and only a small fraction of synapses receive microglial contacts at a given time point. In order to evaluate the influences of microglial contacts in synapse stability, techniques that enable visualization of a large number of synapses and microglial structures are required. CLARITY, a recently-developed technique of tissue processing, enables large volume imaging of the tissue embedded in a substrate with a high reflective index, which enables the use of high NA optics. Here we applied this technique to the study of spatial distributions and morphologies of microglia and synapses in the developing rodent neocortex. Visualization of microglia in a large volume of the neocortex revealed that their spatial distribution is not random. The average

distance between microglial cell body was 50 micrometers and the cells tend to keep their anatomical territory, resulting in formation of the peripheral domain with a reduced density of microglial processes. The border was estimated to be at 15 micrometers away from the cell body. Consistent with this spatial arrangement, the contacts between microglia and dendritic spines were more frequent in the central domain. These data suggest that spatial relationship between dendrites and microglia might have a large impact on the formation of neural circuits by changing the probability of direct contacts between two cell types. Large-volume imaging of CLARITY samples with high resolution optical techniques should be useful for studying the assembly of brain circuits.

SYMPOSIUM 02
Elucidating influence of neuronal activity on CNS myelination

S02-01

Using zebrafish to study how neuronal activity regulates myelination in vivo

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The presence of myelin on axons facilitates rapid nerve impulse conduction, and there is increasing evidence that neuronal activity can modulate myelination, and in so doing fine tune function. Despite recent progress linking regulation of myelination in the central nervous system with important forms of learning and memory, the underlying cellular mechanisms by which activity might regulate myelination by oligodendrocytes in vivo remain largely unclear.

We use zebrafish to study myelinated axon formation, function and repair, and in recent years have generated a suite of transgenic reagents to visualise and manipulate various aspects their biology in vivo. Coupled with the optical transparency and rapid development of the nervous system we can investigate numerous aspects myelination at high-resolution in the living animals in ways that are difficult in other model organisms.

In order to investigate how synaptic vesicle release affects CNS myelination, we have expressed Tetanus Toxin, which cleaves VAMP2 to prevent vesicular release, from either all axon or from individual axons. We find that disruption to synaptic vesicle release from all axons leads to striking reduction in myelinated axon number and that this is primarily due to defects in myelin sheath formation by individual oligodendrocytes. Reduction of synaptic vesicle release from single axons confirms this finding and highlights additional roles in myelin sheath growth. In addition we have taken a pharmacological approach to induce a gain of neuronal function and find corresponding increase in myelin sheath production by individual oligodendrocytes. We have characterised the activity of the myelinating processes and myelin sheaths of oligodendrocytes using the genetically encoded calcium indicator, GCaMP6s and are currently testing how various activity signatures relate to myelination fate and myelin sheath growth.

In this presentation I will also outline our plans to positively and negatively regulate the function of individual neurons over extended periods of time and along mature myelinated axons. Together this work aims to provide insight into a potentially very important form of activity dependent nervous system plasticity.

S02-02

Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain

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Myelination of the central nervous system requires the generation of functionally mature oligodendrocytes from oligodendrocyte precursor cells (OPC). Electrically active neurons may influence OPC function and selectively instruct myelination of an active neural circuit. We use optogenetic stimulation of premotor cortex in awake, behaving mice to demonstrate that neuronal activity elicits a mitogenic response of neural progenitor cells and OPCs, promotes oligodendrogenesis and increases myelination within the deep layers of the premotor cortex and subcortical white matter. We further show that this neuronal activity-regulated oligodendrogenesis and myelination is associated with improved motor function of the corresponding limb. Oligodendrogenesis and myelination appear necessary for the observed functional improvement, as epigenetic blockade of oligodendrocyte differentiation and myelin changes prevents the activity-regulated behavioral improvement.

S02-03

The effects of pharmacogenetic manipulation of neuronal activity on oligodendrocyte turnover and myelination in the developing mouse brain

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Myelination in vertebrates is critical not only for rapid conduction of electrical signals along axons, thus allowing for larger, more complex brains, but is also integral for supporting normal axonal health and function. Myelination is tightly regulated by numerous extracellular signals that converge on specific transcription factors to enact changes in the differentiation state and function of the cell that produces myelin, the oligodendrocyte (OL). It has previously been shown that directly stimulating neuronal activity enhances myelination, and conversely that inhibiting activity decreases myelination. However, the cellular dynamics underlying activity-dependent myelination are poorly understood. To investigate this question, we first employed a pharmacogenetic approach utilising the hM3Dq DREADD (designer receptor exclusively activated by designer drug) to chronically stimulate activity in a subpopulation of callosal axons in the somatosensory cortex (S1). Twelve post-natal day 5 (P5) mice were injected with an AAV1/2-hM3Dq virus into S1 and given daily injections of either the hM3Dq-specific agonist clozapine-N-oxide (CNO) or saline for 1 week from P19. Likewise, to study the effects of reducing neuronal activity we overexpressed the inward rectifying K^+ channel Kir2.1 by either electroporating a Kir2.1-GFP construct or GFP control construct into S1 of E14.5 mouse embryos (n = 10/condition). Stimulating neuronal activity in S1 for seven days (P19+7) increased proliferation of oligodendrocyte precursor cells (OPCs) (216.9 ± 23.3 vs 146.7 ± 13.0 $PDGF^+$ EdU^+ cells/mm^2; CNO vs Saline; mean ± SEM; $p < 0.01$) without altering overall OPC density. OPC differentiation was also augmented by stimulating activity, resulting in an increased density of mature OLs (1744.8 ± 113.7 vs 1418.8 ± 57.3 CC1+ cells/mm^2; CNO vs Saline; mean ± SEM; $p < 0.01$). Conversely, Kir2.1-mediated inhibition of neuronal activity significantly increased OPC density, but did not alter the overall density of mature OLs. Confocal microscopy revealed that stimulating activity significantly increased the density of myelin basic protein (MBP) profiles that were double-labelled for GFP compared to controls. On the other hand, attenuating activity with Kir2.1 resulted in the opposite effect, with fewer GFP/MBP-co-labelled axons compared to controls. This study demonstrates that neuronal activity plays an important role in regulating myelination by modulating both the proliferation of OPCs and their subsequent differentiation into mature myelinating OLs.

S02-04

Neuronal activity regulates remyelination via glutamate signalling to oligodendrocyte progenitors

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Myelin is essential for normal brain function, as it enables fast information transmission and trophic support for axons. Its importance is evident in diseases, such as spinal cord injury and multiple sclerosis, where it is lost or damaged, as this leads to mental and/or physical disability. During

development, oligodendrocyte precursor cells (OPCs) differentiate into myelinating oligodendrocytes, which myelinate axons. In white matter diseases, adult OPCs rapidly respond to demyelinating lesions and differentiate to replace lost myelin sheaths to recover fast axonal transmission; however, this repair process often fails. To promote endogenous myelin repair it is essential to understand the mechanism that can regulate remyelination.

Glutamate and electrical activity influence OPC differentiation and myelination in normal development. OPCs receive synaptic input from unmyelinated axons, possibly to initiate myelination, and both OPCs and mature oligodendrocytes respond to glutamate via AMPA and NMDA receptors. However, little is known about the role different OPCs' glutamate receptors play in remyelination.

Therefore we have examined the role of glutamate signalling in remyelination following experimental demyelination in the adult rodent CNS. We voltage-clamped OPCs in brain-slices of adult rat cerebellar peduncle containing focal areas of primary demyelination and post-identified these by NG2-immunolabelling. OPCs recruited to the demyelinated lesion expressed AMPA/Kainatereceptors at the peak of the OPCs proliferation (5-10 days post-lesion) but lacked NMDA receptor expression at this time point.

The demyelinated axons continued to propagate action potentials, but with latencies similar to those in unmyelinated axons during development. Critically, the demyelinated axons established synapses with OPCs expressing voltage-gated sodium currents. These synaptic inputs had identical decay times to those recorded during developmental myelination. Pharmacological manipulation of neuronal activity or glutamate signalling blocked remyelination by affecting OPCs differentiation.

These findings reveal how neuronal activity and release of glutamate instruct OPCs, via AMPA receptors, to differentiate into new oligodendrocytes that restore myelin and recover lost function.

SYMPOSIUM 03
Astrocyte gliotransmission, volume signalling and water homeostasis

S03-01

Metabolic regulation of vesicular glutamate release from cultured astrocytes

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Astrocytes have a prominent role in brain physiology and pathophysiology. In addition to maintaining blood flow, metabolic and ionic homeostasis, they have capability of signaling to adjacent neurons by releasing glutamate via process of regulated exocytosis. Astrocytes synthesize glutamate *de novo* owing to pyruvate entry to the citric acid cycle via pyruvate carboxylase. Pyruvate is sourced from the utilization of two metabolic fuels, glucose and lactate. Glucose can be polymerized to glycogen and stored as fuel within astrocytes and/or lysed to pyruvate, while lactate can be converted to pyruvate. To that end, we investigated the role of the above energy sources, glycogen, glucose and lactate, in exocytotic glutamate release from astrocytes. We used purified primary astrocyte cultures acutely incubated (1 hour) in glucose and/or lactate-containing media. We used mechanical stimulation, known to increase intracellular calcium levels and cause exocytotic glutamate release. Using single cell fluorescence microscopy, we monitored stimulus-induced intracellular calcium responses as well as glutamate release to the extracellular space. Our data indicate that glucose, either taken-up from media or mobilized from the glycogen storage, sustained glutamate release, while the availability of lactate significantly reduced the release of glutamate from astrocytes. Based on further pharmacological manipulation, it appears that lactate caused metabolic changes consistent with an increased synthesis of fatty acids. The above metabolic and functional changes were corroborated by

tandem mass spectrometry proteomics analysis which confirmed appropriate altered protein expression. These findings support the notion that the availability of energy sources and metabolic milieu play a role in glial-neuronal interactions and modulation of synaptic activity in health and disease.

S03-02

Astrocytic gliotransmitter vesicles and their interaction with the plasmalemma

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Exocytosis is a universal process, important for communication between cells. However, the anatomy of vesicles and their interaction with the plasma membrane remain unclear, mainly due to experimental inaccessibility. In the central nervous system, not only neurons but also astrocytes utilize vesicular transmitter release, or gliotransmission. The existence of this process was discussed in a recent issue of *Neuron* (Sloan and Barres, 2014, *Neuron*).

Perhaps the strongest *in vivo* evidence in support of gliotransmission comes from studies of a transgenic mouse line in which vesicular release can be specifically inhibited in astrocytes. In these mice, the formation of the SNARE complex between vesicles and the plasma membrane is inhibited by the expression of a dominant-negative domain of the vesicle-associated membrane protein 2 (VAMP2) protein (dnSNARE), which interferes with endogenous VAMP2 expression and thus prevents VAMP2-mediated membrane fusion (Sloan and Barres, 2014). However the mechanism of action of this peptide is unknown.

In this presentation we systematically determined astrocytic vesicle anatomy using super-resolution STED and SIM microscopies. We found that amino acid and peptide gliosignals reside in smaller vesicles (70 nm diameter), whereas ATP is present in larger vesicles (200 nm diameter). To study the interaction of these vesicles with the plasma membrane, we measured membrane capacitance with a high-resolution approach (Rituper *et al*., 2013, *Nature Protocols*). We found that smaller vesicles exhibit mainly transient (kiss-and-run) fusion, which is enhanced upon stimulation with ATP. Larger vesicles, on the other hand, proceed from a narrow fusion-pore state to full fusion exocytosis upon stimulation.

To test whether the density of SNARE molecules determines the vesicle size-dependent mode of exocytosis, we disrupted the integrity of these proteins by botulinum neurotoxins D and E and by dnSNARE peptides, while monitoring unitary exocytotic events. We found that such events are dependent on Ca^{2+} and SNARE protein; however, the vesicle size-associated mode of exocytosis is not altered if the functional integrity of the SNARE proteins is affected.

This work contributes to our understanding of regulated exocytosis by showing (1) that the post-fusion role of SNARE proteins in regulating fusion pore widening is independent of vesicle diameter and (2) that dnSNARE peptide stabilizes the fusion-pore in a narrow, release-unproductive, state. These findings will contribute to the debate on gliotransmission.

S03-03

Lactate transport and signaling in the brain: potential therapeutic targets and roles in body-brain interaction

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Lactate acts as a "buffer" between glycolysis and oxidative metabolism. In addition to being exchanged as a fuel by the monocarboxylate transporters (MCTs) between cells and tissues with different glycolytic and oxidative rates, lactate may be a "volume transmitter" of brain signals. According to some, lactate is a preferred fuel for brain metabolism. Immediately after brain activation, the rate of glycolysis exceeds oxidation, leading to net production of lactate. At physical rest, there is a net efflux of lactate from the brain into the blood stream. But when blood lactate levels rise, such as in physical exercise, there is net influx of lactate from blood to brain, where the lactate is used for energy production and myelin formation. Lactate binds to the lactate receptor GPR81 aka HCAR1 on brain cells and cerebral blood vessels, and regulates the levels of cAMP. The localization and function of HCAR1 and the three MCTs (MCT1, MCT2 and MCT4) expressed in brain constitute the focus of my talk. They are possible targets for new therapeutic drugs and interventions. I propose that lactate actions in the brain through MCTs and the lactate receptor underlie part of the favourable effects on the brain resulting from physical exercise.

S03-04

Role of astrocytes in volume and water homeostasis

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Aquaporin-4 (AQP4) is one of the most abundant molecules in the brain and is particularly prevalent in astrocytic membranes at the blood-brain and brain-liquor interfaces. While AQP4 has been implicated in a number of pathophysiological processes, its role in brain physiology has remained elusive. Only recently has evidence accumulated to suggest that AQP4 is involved in such diverse functions as regulation of extracellular space volume, potassium buffering, cerebrospinal fluid circulation, interstitial fluid resorption, waste clearance, neuroinflammation, osmosensation, cell migration, and Ca(2+) signaling. AQP4 is also required for normal function of the retina, inner ear, and olfactory system. An overview will be provided of the physiological roles of AQP4 in brain and of the growing list of data that emphasize the roles of astrocytes in water and volume homeostasis.

SYMPOSIUM 04
Schwann cell-axon interaction controls peripheral nerve development and disease

S04-01

Nuclear control of axonal sorting in nerve development and dysmyelinating neuropathy

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Peripheral nerve development is a complex multistep process in which large myelinated and small unmyelinated fibers are formed. Schwann cells undergo controlled proliferation, differentiation and interaction with the surrounding axons in order to form mature nerve. This process, termed radial axonal sorting, is originated by axonal and extracellular matrix (ECM) signals, and mediated within Schwann cells by an increasing number of cytoplasmic molecules. How ECM and axonal signals are integrated to orchestrate Schwann cell proliferation, survival, and differentiation to successfully sort axons is still mostly obscure. Moreover, this crucial morphogenetic step is a prerequisite for myelination and differentiation of Remak fibers, and is arrested in human diseases due to mutations in genes coding for ECM and linkage molecules.

We recently contributed to understand the Schwann cell nuclear control of the radial sorting of axons. We identified the nuclear molecule Jun activation domain-binding protein 1 (Jab1) as a key factor downstream to signals originated by ECM and axons. Jab1 controls Schwann cell number and differentiation during nerve development and the axonal sorting process. We also revealed that Jab1 acts through the control of the cyclin inhibitor p27, and that the disruption of the Jab1 function results

in dysmyelinating neuropathy and progressive axonal degeneration. The modulation of p27 levels in Jab1 null mutants can rescue axonal sorting defect, dysmyelination and axonal degeneration.

S04-02

Spatial mapping of polarized axo-glial interactions reveals new molecules involved in myelination

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Interactions between glial cells and neurons or other cells promote juxtacrine signals that are difficult to capture in the complexity of the nervous system. For example, contact between axons and Schwann cells triggers signals required for radial sorting and myelination. Failure in this interaction causes dysmyelination and axonal degeneration, but despite its importance, few molecules at the neuronal-glial surface are known. To identify novel molecules in neuro-glial interactions, we modified the “pseudopodia” sub-fractionation system and isolated the projections that glia extend when they receive juxtacrine signals from axons. By proteomics we identified the signaling networks present at the glial leading edge, and a family of proteins, the Prohibitins, that was previously unknown in Schwann cells. We show that Glial-specific deletion of Prohibitins in mice impairs neuro-glial interactions and myelination. We thus describe and validate a novel method for modeling morphogenesis and profiling juxtacrine signaling and describe a novel class of molecules in myelination.

S04-03

The functional organization of myelinated axons and the role of LGI proteins

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The stereotypic distribution of voltage-gated sodium and potassium channels in myelinated axons allows rapid action potential impulse propagation along the length of the axon. The distribution of these channels is dictated by the myelin sheath, which is produced and maintained by glial cells. While much is known about the cellular and molecular mechanisms that direct accumulation of voltage-gated sodium channels to the node of Ranvier, relatively little is known about the mechanisms that govern accumulation of shaker type voltage-gated potassium channels at the juxtaparanodal membrane under the myelin sheath. The accumulation of these potassium channels (Kv1) does depend on the cell adhesion molecules Caspr2 and Tag-1 and their interaction with the axonal cytoskeleton but how this contributes to Kv1 clustering is still unclear. We found that members of the LGI family of secreted proteins and their axonal receptors are required for both myelination and subsequent Kv1 clustering. We will present our most recent work that sheds light on the mechanism of myelination and axonal domain organization and the role of LGI and ADAM proteins in these processes.

S04-04

Directing axonal regrowth following nerve injury

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The peripheral nervous system has remarkable regenerative capacities following injury including the ability to repair a fully cut nerve. This requires the regrowth of axons to their targets in the absence of the guidance signals present during development. In my presentation, I will present work which shows the complex multicellular response required to create this new tissue within an adult organism. In this process, macrophages, endothelial cells, Schwann cells and fibroblasts act togther to ensure the

correct guidance of axons back to their targets. This work has implications for improving nerve repair as well as for the spread of tumour cells within this tissue.

SYMPOSIUM 05
Systematic approaches to glia

S05-01

What do reactive astrocytes do?

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Astrocytes undergo profound changes in morphology and gene expression in response to brain injury and disease. But whether reactive astrocytes are harmful or helpful has been unclear. We recently found that the genes induced in reactive astrocytes depends on the nature of the inducing injury. After ischemia, reactive astrocytes upregulate neurotrophic factors suggesting they may be beneficial, whereas after systemic injection of lipopolysaccharide (LPS) they strongly upregulate multiple complement cascade components needed to drive synapse destruction suggesting they may be detrimental. These findings suggest that, like macrophages which exist on a spectrum from bad (M1) to good (M2) states, reactive astrocytes also exist in bad (A1) and good (A2) states. Here we show that LPS-induced M1 microglia are sufficient to induce A1 reactive astrocytes. M1 microglia do this by releasing IL1α, TNFα and C1q, which together are sufficient to induce A1 (bad) reactivity in purified astrocytes within 24h and are all required for M1 microglia to induce the A1 state. Using IL1α, TNFα and C1q together, allowed us to create the first defined serum-free cultures of pure A1 reactive astrocytes enabling us to investigate their function. By directly comparing the function of normal astrocytes with A1 astrocytes in vitro, we found that A1 astrocytes are unable to promote neuronal survival, axon outgrowth, synapse formation or synapse function, and have lost the ability to phagocytose synaptosomes and myelin debris. In addition to loss of their normal functions, A1 reactive astrocytes gained a powerfully neurotoxic function, releasing a toxic protein that specifically induces apoptosis of neurons and oligodendrocytes. Drugs that prevent the formation of A1 reactive astrocytes or inhibit this toxic protein may have great potential to treat neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Multiple Sclerosis.

S05-02

Proteomic approach to myelin-related neuropathology

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Our normal motor, sensory, and cognitive abilities require fast nerve conduction, which is facilitated by the myelination of neuronal axons. We are interested in the functional cooperation between neurons and myelinating glial cells. The axon/myelin-unit becomes impaired in myelin-related disorders and upon normal aging. Striking pathological commonalities are observed in various myelin-related neurological disorders, including structurally impaired myelin, hypomyelination, axonopathy, and neuroinflammation. It has thus been difficult to explain particular aspects of neuropathology by mutations affecting individual myelin proteins. We have hypothesized that such mutations cause secondary changes of the protein composition of myelin, which in turn might trigger the observed impairments. I will present a systematic assessment of the neuropathology and the myelin proteome in three mouse models of complex myelin-related disorder. This work has enabled us to correlate distinct neuropathological features with alterations of the myelin proteome. In this context I will discuss the identification of a previously unrecognized cytoskeletal filamentous structure in the central nervous system, which prevents pathological myelin outfoldings by scaffolding the innermost myelin layer. Our data imply that myelin outfoldings contribute to declining capabilities as seen in neurological disorders and upon normal ageing.

S05-03

Integrative -omics analyses uncovers inflammatory lipid cascades in adrenoleukodystrophy

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X-linked adrenomyeloneuropathy (AMN) is an inherited neurometabolic disorder caused by malfunction of the ABCD1 gene, characterized by slowly progressing spastic paraplegia affecting axonal corticospinal tracts, and adrenal insufficiency. AMN is the most common phenotypic manifestation of adrenoleukodystrophy (X-ALD). In some cases, an inflammatory cerebral demyelination occurs associated to poor prognosis in cerebral AMN (cAMN). Though ABCD1 codes for a peroxisomal transporter of very long-chain fatty acids, the molecular mechanisms that govern disease onset and progression, or its transformation to a cerebral, inflammatory demyelinating form, remain largely unknown. Here we used an integrated -omics approach to identify altered network dynamics characteristic of, and possibly driving the disease. We combined an untargeted metabolome assay of plasma and peripheral blood mononuclear cells (PBMC) of AMN patients, which used liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-Q-TOF), with a functional genomics analysis of spinal cords of Abcd1- mouse. The results uncovered altered nodes in lipid-driven proinflammatory cascades, such as glycosphingolipid and glycerophospholipid synthesis, governed by the beta-1,4-galactosyltransferase (B4GALT6), the phospholipase 2γ (PLA2G4C) and the choline/ethanolamine phosphotransferase (CEPT1) enzymes. Confirmatory investigations revealed a non-classic inflammatory profile, and a previously unreported connection between ABCD1 dysfunction, glyco- and glycerolipid-driven inflammatory signaling and a fine-tuned inflammatory response underlying a disease considered non-inflammatory.

S05-04

Remyelination: drug discovery and pharmacology

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Progressive phases of multiple sclerosis (MS) are associated with inhibited differentiation of the progenitor cell population required for remyelination and disease remission. To identify selective inducers of oligodendrocyte differentiation, we developed an imaging-based assay using primary rat optic nerve-derived progenitor cells and used this to screen a collection of ~200,000 structurally diverse small molecules. Amongst the most effective compounds identified were anti-muscarinic drugs, which were subsequently found to significantly decrease clinical severity in the experimental autoimmune encephalomyelitis (EAE) model of relapsing-remitting MS when administered alone or in combination with approved immunosuppressive treatments for MS. Evidence from a Cuprizone-induced model of demyelination, *in vitro* and *in vivo* T cell assays, and EAE adoptive transfer experiments indicate that the observed efficacy of this drug results directly from an enhancement of remyelination rather than immune suppression. A potential limitation to the direct clinical application of these results is the on-target dose limiting toxicity associated with anti-muscarinic drugs. Progress towards the identification of optimal anti-muscarinic drugs for the treatment of MS and clinical considerations for this approach will discussed. Further, novel mechanism of action small molecule promyelinating drug candidates will be described.

SYMPOSIUM 06
Microglial dynamics in the healthy and diseased brain

S06-01

The origins of microglia

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Microglia are the specialised macrophages of the brain. In common with macrophages throughout the body, their appearance and differentiation is controlled by signalling through the CSF1 receptor. In adult mice, they appear long-lived and do not require replenishment form circulating monocytes. In mouse embryos, Csf1r+ cells first appear in the yolk sac, and infiltrate the developing brain. An emerging literature has concluded that the yolk sac is the exclusive source of microglia in the adult brain in the mouse. However, that conclusion is based upon experimental manipulations that probably disturb CSF1 homeostasis, and do not necessarily reflect the normal developmental path. In mice at least, there is a large expansion of microglial numbers around the perinatal period, and early studies provided clear evidence especially in the retina, for migration of monocytes from the blood. We have previously generated novel reporter genes in mice based upon the Csf1r promoter and have used them to trace the appearance of microglia during development. To address the origins of microglia and tissue macrophages we have now investigated the chick system, which permits convenient manipulation by microinjection during development. We have produced CSF1R reporter transgenic birds, anti-CSF1R antibodies and recombinant chicken CSF1. As in the mouse, CSF1R+ cells first appear in the yolk sac. Prior to the onset of definitive haemopoiesis, we isolated yolk-sac derived blood cells from EGFP reporter birds. These cells are capable of massive expansion in CSF1 in vitro. When transplanted into EGFP-negative recipients of the same age, they populated microglial and peripheral macrophage pools all over the embryo. However, all EGFP+ cells were gone by the time of hatch. By contrast, when we transplanted EGFP+ bone marrow cells from hatchling donors into early embryos, all macrophage pools including microglia, were similarly populated. When transplant recipients were taken to hatch, they retained large proportions of EGFP+ macrophages into adulthood. There was no chimerism observed in non-macrophage haemopoietic compartments, suggesting the existence of a specialised bone marrow macrophage progenitor cell. We investigated the sensitivity of embryonic macrophages to variation in the available CSF1 by injecting a novel CSF1-Fc protein in ovo. The treatment caused a massive expansion of the macrophage populations all over the embryo. The data from the chick system support the view that microglia derived from yolk sac are replaced during development by cells of definitive haemopoietic origin. In this respect, they resemble most, if not all, other tissue macrophage populations.

S06-02

Microglia in the universe of myeloid cells in the CNS

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Microglia are immunocompetent macrophages of the brain that continuously scrutinize but are in turn also exposed to their environment. They colonize the developing neuroectoderm already during embryogenesis shaping the neuronal network by immune-mediated mechanisms, and then dramatically change their repertoire in the matured brain during health and inflammatory conditions. These dramatic and sometimes sudden changes require this long-lived cell type to carry out multifaceted adaptive changes of high accuracy. Deciphering microglia responsiveness to divergent stimuli is central for manipulating this immune cell type and to potentially reducing disease burden. Major advances in our understanding of microglia biology were obtained in the last years, especially by combining novel gene targeting techniques with advanced gene profiling. Here we discuss new aspects of myeloid cell biology in general with special emphasis on the shifting role of microglia during establishment and protection of brain integrity.

S06-03

Microglia polarization: myths and realities

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Myelomonocytic cells are classified based on a combination of surface markers and cytokines into inflammatory, or M1-type and anti-inflammatory or M2-type. Multiple subtypes of M2 macrophages have also been described. However, this classification has been mostly based on in vitro experiments in which bone marrow derived macrophages are treated with pharmacological amounts of cytokines. In contrast, the ability of yolk sac derived macrophages to polarize in a similar fashion has been largely assumed, but not demonstrated experimentally. Microglia represent prototypical tissue-resident, yolk sac macrophages. Using genetic methods to distinguish microglia from infiltrating macrophages, we have investigated gene expression in both bulk-purified and individual microglial cells at steady state and at different clinical stages of a neurodegenerative (ALS) and a neuroinflammatory (EAE) disease in murine models. We found that microglia adopt specific states in the two diseases, but that none of these states reflects what would be predicted by the M1/M2 classification.

S06-04

Microglial proliferation in chronic neurodegeneration

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An important aspect of chronic neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's and prion disease, is the generation of an innate inflammatory response within the central nervous system (CNS). Microglial cells play a key role in the development and maintenance of this inflammatory response, showing enhanced proliferation and morphological activation. Using a laboratory model of chronic neurodegeneration (ME7 murine model of prion disease), we studied the time-course and regulation of microglial proliferation. Our results show that resident microglial cells have an increased proliferation rate during the development of the disease, leading to a significant increase in the population, without a contribution from circulating cells, as detected by using CCR2-/- mice. We have identified the CSF1R pathway as the main regulator of microglial proliferation in murine and human chronic neurodegeneration. The specific inhibition of the activity of CSF1R leads to an amelioration of the behavioural symptoms and an extension of the survival of prion diseased mice. We also found a correlation of our results with the scenario present in chronic human neurodegenerative conditions variant Creutzfeldt-Jakob Disease (vCJD) and Alzheimer's disease. Our results demonstrate that microglial proliferation is an important feature of the evolution of chronic neurodegenerative disease, with direct implications for understanding the contribution of the CNS innate immune response to disease progression.

SYMPOSIUM 07
Oligodendrocyte differentiation in development and pathology

S07-01

Demyelination-induced changes in oligodendrocyte progenitor cells in the adult CNS

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While the physiological function of oligodendrocyte progenitor cells (OPCs) in the normal CNS remains uncertain, it is well established that adult OPCs (aOPCs) are primarily responsible for generating new oligodendrocytes (OLs) and the restoration of myelin sheaths following demyelination. At least three distinct sequential phases of remyelination are identifiable: first, a process of activation, in which aOPC in the vicinity of the injury change their shape and gene expression profile; second, aOPC recruitment into and within the demyelinated area by migration and proliferation; third, differentiation of the recruited aOPCs into mature myelin sheath forming oligodendrocytes.

To gain insight into these different steps of the repair process, we used purified oligodendrocyte progenitor cells (OPCs) from adult murine normal and demyelinated CNS, experimental models of demyelination and post-mortem MS brain tissue samples. We found that adult OPCs have a transcriptome more similar to that of oligodendrocytes than to neonatal OPCs, but revert to a neonatal-like transcriptome when activated. We then demonstrated that part of the activation response involves increased expression of two genes of the innate immune system, *IL1β* and *CCL2*, which enhance the mobilization (chemokinetic effect) of OPCs. In parallel, we showed that recruitment (directional migration) of activated OPC to the demyelinated areas, depends on different guidance cues: whereas Netrin1 and semaphorin 3A are repellent to OPCs, Semaphorin 3F is attractant. We further demonstrated that this effect on OPC recruitment influences remyelination rate.

Our results further decipher the mechanisms involved in these different steps of the remyelination process, paving the way for pre-clinical studies aimed at enhancing remyelination.

S07-02

Wrapped up tight: netrin function in mature myelin

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Paranodal axoglial junctions are essential for the segregation of myelinated axons into distinct domains and the efficient conduction of action potentials. Recent studies have highlighted paranodes as a site of potential vulnerability and early damage in patients with multiple sclerosis; however, the mechanisms required to organize and maintain paranodal junctions are not fully understood. The secreted protein netrin-1 is enriched at paranodes and previous studies using organotypic slice cultures have suggested that netrin-1 function is required for paranode maintenance. Both neurons and oligodendrocytes express netrin-1 and netrin receptors, but it had not been determined whether paranode maintenance requires expression by the oligodendrocytes, the axon, or both. To address the specific contributions of netrin-1 and netrin receptors made by oligodendrocytes to myelination, myelin maintenance, and remyelination, we have selectively manipulated expression in myelinating oligodendrocytes. Disrupting netrin function resulted in the progressive disorganization of paranodes and compact myelin during aging, revealing essential contributions to myelin maintenance. Furthermore, netrin receptor conditional knockout mice display decreased conduction velocity and develop balance and coordination deficits. We conclude that netrin function is required for the maintenance and stability of myelin *in vivo*, which is essential for proper signal conduction in the CNS. Importantly, identifying and understanding the molecular mechanisms that promote the stability and maintenance of myelin will facilitate the development of new therapeutic strategies to treat and ultimately prevent demyelinating disease.

S07-03

mTOR signaling in oligodendrocyte progenitor cell differentiation and myelination

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Understanding how intracellular signaling pathways connect extracellular signals with transcription regulators of oligodendrocyte differentiation and myelination is critical to elucidating how OPCs are inhibited from promoting remyelination in a lesion environment. We have shown in prior studies that the mTOR signaling pathway is critical for OPC differentiation and developmental myelination both in

vitro and in vivo [1, 2]. To further understand the mechanisms for how mTOR regulates oligodendrocyte differentiation and myelination, we are investigating downstream targets of mTOR including transcriptional regulators and proteins involved in morphological differentiation of the lineage. Preliminary studies also support the conclusion that the mTOR pathway functions in OPC differentiation and remyelination after a demyelinating insult.

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S07-04

Transgenic Xenopus: a model of inducible demyelination and remyelination

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In the *MBP-GFP-NTR Xenopus laevis* transgenic line, conditional ablation of myelin-forming oligodendrocytes can be achieved and remyelination monitored. In this transgenic line the transgene is driven by the proximal portion of myelin basic protein (MBP) regulatory sequence, and is specifically expressed in myelin forming oligodendrocytes. The transgene protein is formed by GFP reporter fused to the *E. coli* nitroreductase (NTR) selection enzyme. This enzyme converts the innocuous pro-drug metronidazole (MTZ) to a cytotoxin. Ablation of oligodendrocytes by MTZ treatment of the tadpole induced demyelination. Myelin debris are eliminated by microglial cells identified by isolectinB4 labeling. After cessation of MTZ treatment, remyelination proceeded spontaneously. We questioned the origin of remyelinating cells. Our data suggest that, $Sox10^+$ oligodendrocyte precursor cells (OPCs), which are already present in the optic nerve prior to the experimentally induced demyelination, are responsible for remyelination. In addition, spontaneous remyelination can be pharmacologically accelerated. We will present data showing that our *Xenopus* model can be used to screen *in vivo* remyelinating compounds.

SYMPOSIUM 08
Role of glial cells in rare diseases

S08-01

Astrocyte dysfunctions in Huntington's disease model mice

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Huntington's disease (HD) is characterized by striatal medium spiny neuron (MSN) dysfunction, but underlying mechanisms remain unclear. We recently explored roles for astrocytes (1), which display mutant huntingtin in HD patients and mouse models. We found that symptom onset in R6/2 and Q175 HD mouse models was not associated with classical astrogliosis, but was associated with decreased Kir4.1 K^+ channel functional expression, leading to elevated *in vivo* levels of striatal extracellular K^+, which increased MSN excitability *in vitro*. Viral delivery of Kir4.1 channels to striatal astrocytes restored Kir4.1 function, recovered aspects of MSN dysfunction, prolonged survival and attenuated some motor phenotypes in R6/2 mice. These findings indicated that components of altered MSN excitability in HD may be caused by heretofore unknown astrocyte disturbances.

Astrocytes display dynamic intracellular Ca^{2+} fluctuations, which are considered important for a variety of physiological processes (2). In the present study, we used genetically encoded Ca^{2+} and glutamate indicators and mouse models of Huntington's disease to explore when astrocytes display Ca^{2+} signaling in the striatum. In healthy mice, striatal astrocytes displayed numerous spontaneous Ca^{2+} elevations, but failed to respond to stimulation of cortical inputs. In R6/2 mice, spontaneous Ca^{2+} elevations were significantly reduced, but astrocytes displayed robust Ca^{2+} elevations during stimulation of cortical inputs. These action-potential evoked Ca^{2+} responses were mediated by glutamate receptors, accompanied by prolonged glutamate levels in the extracellular space and reproduced in healthy mice by blocking Glt1 glutamate transporters. Moreover, dysfunctional astrocyte Ca^{2+} signaling in R6/2 mice was significantly rescued by astrocyte expression of Kir4.1 via a mechanism involving Glt1, whose expression was reduced in HD but rescued by Kir4.1 (1). Thus, astrocyte engagement in the striatal microcircuitry dramatically changes in HD and is regulated by its key homeostatic functions. Taken together, our data suggest that defects in neurodegenerative diseases may be remedied by targeting astrocyte-mediated homeostasis.

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S08-02

Astrocytes may be behind the pathogenesis of 22q11

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The onset and etiology of schizophrenia has been associated with a wide range of genetic and epigenetic factors including perturbations in mitochondrial function and oxidative stress responses. The 22q11 deletion syndrome (DS) is one of the highest genetic risk factors for schizophrenia and includes the gene proline dehydrogenase (PRODH) coding for mitochondrial enzyme regulating L-proline (L-Pro). Aim of this work is to elucidate the role of PRODH in the pathophysiology of 22q11DS. The expression of PRODH is typically ascribed to proliferating tissues (Polyak et al., *Nature*, 1997) where it regulates cell proliferation and metabolism (Liu et al., *PNAS*, 2012). We and others have found that PRODH expression in the brain is limited to postnatal development, the temporal period commonly associated with gliogenesis. Interestingly, we have found the PRODH expression in the astrocytes seems to be limited to the prefrontal cortex (PFC). These astrocytes, similar to the proliferating cells, showed enhanced glycolytic rates which could render astrocytes more susceptible to mitochondrial perturbations. Indeed, in astrocytes of PRODH-deficient mice, respiration, ATP levels and mitochondrial transmembrane potential (deltaPsi) were impaired. These mice showed also cognitive impairments associated with PFC. We concluded that PRODH expression during postnatal development is necessary to maintain a proper mitochondrial function. *Support contributed: NCCR Synapsy to P. Bezzi*

S08-03

Glial cell toxicity towards motor neurons in ALS

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For the last century, research on neurological disorders has focused on the most prominent affected cell type - the neurons. However, with increasing knowledge of the diverse physiological functions of glial cells, their impact on neurological disorders has become more evident. Therefore, many conditions appear to have more complex origins than initially thought. Since neurological pathologies are often sporadic with unknown etiology, animal models are difficult to create and might only reflect a small portion of patients in which a mutation in a gene has been identified. Therefore, reliable *in vitro* systems to study these disorders are urgently needed. They might be a pre-requisite for improving our understanding of the disease mechanisms as well as for the development of potential new therapies. In this talk focused on the motor neuron disease Amyotrophic Lateral Sclerosis, a summary of the function of different glial cell types in the disease will be discussed. We will then describe different types of culture systems to model non-cell autonomous interactions *in vitro* and evaluate advantages and disadvantages.

S08-04

Functional deficits of the astrocytes in Amyotrophic Lateral Sclerosis

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Collective evidence indicates that motor neuron degeneration in Amyotrophic Lateral Sclerosis (ALS) is non-cell-autonomous and requires the interaction with the neighboring astrocytes. Astrocytes can hurt motor neurons by secreting neurotoxic factors, but they can play deleterious roles also by losing functions that are supportive for neurons.

Recently, we reported that stimulation of inositol 1,4,5 triphosphate (IP_3)-generating group I metabotropic glutamate receptors in ALS astrocytes triggers abundant and persistent elevations of intracellular Ca^{2+} concentrations in the absence of spontaneous oscillations. This correlates with mitochondrial disarrangement and cell death in subsets of astrocytes. The interaction of IP_3 receptors with the anti-apoptotic protein Bcl-X_L was previously described to prevent cell death by generating pro-survival Ca^{2+} oscillations. In ALS astrocytes, we found that the sole BH4 domain of Bcl-X_L, fused to the protein transduction domain of the HIV-1 TAT protein (TAT-BH4), is sufficient to restore sustained Ca^{2+} oscillations and cell death resistance. Furthermore, chronic treatment of ALS mice with the TAT-BH4 peptide exerts a positive impact on the disease manifestations. Besides, we demonstrate that ALS astrocytes aberrantly respond to the neuroinflammatory microenvironment, exhibiting a functional impairment of their neurotrophic properties.

SYMPOSIUM 09
Chromatin remodeling in myelin development and nerve regeneration

S09-01

The role of chromatin remodeling in myelinating glia

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Myelination is a vertebrate invention to allow accelerated nerve conduction. Its establishment and maintenance depend on a unique regulatory network made up of transcription factors, epigenetic regulators and microRNAs. Transcription factors have been extensively studied in the last years. Thus it is known, for instance, that the transcription factor Sox10 is needed in both oligodendrocytes and Schwann cells for progenitor cell survival, lineage progression, terminal differentiation and myelin gene expression, and cooperates during the process with other transcription factors such as Olig2 and Myrf, and the mediator complex. In contrast, much remains to be learnt about the role of epigenetic factors. This presentation will focus on the role of two chromatin remodeling complexes, the BAF complex and the p400 complex for development and differentiation of myelinating glia. Special emphasis will be placed on similarities and differences between Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system, as well as on the functional interaction of the chromatin machinery with central glial transcription factors such as Sox10.

S09-02

Chromatin dynamics in Schwann cells after nerve injury

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Schwann cells undergo substantial gene expression changes after nerve injury, and recent studies have highlighted their importance in supporting axon regeneration. Significant progress has been made in identifying specific transcriptional regulators that support this transition. However, gene expression changes during injury require not only individual transcription factors, but also their cognate enhancers that integrate the actions of multiple transcription factor inputs to regulate associated genes. These distal enhancer elements are integration hubs for signaling pathways activated by extracellular signals. Recent genomics studies using chromatin immunoprecipitation (ChIP-seq) have offered tools to assess not only transcription factor binding but also enhancer activation status in specific tissues in a stage-dependent manner. To examine how peripheral nerve injury alters the global transcription program, we have determined injury induced changes in a mark of active enhancers, histone H3K27 acetylation. Analysis of both injury-induced and injury-suppressed enhancers is consistent with the role of known transcriptional regulators of injury, such as c-Jun, but also reveal some novel factors and their role in nerve injury. Moreover, our results identify dynamic enhancer changes that are likely involved in altering gene expression after injury.

While transcriptional regulation plays a major role, it is clear that chromatin remodeling complexes and epigenomic modulation also plays a role in gene expression changes. We have investigated the function of the Polycomb Repressive Complex 2 (PRC2) in Schwann cell development by analyzing the effects of a Schwann cell-specific deletion of the Eed subunit. This complex is required for repressive methylation on H3K27. Surprisingly, we find that this complex is not required for postnatal development of myelin, but inactivation of PRC2 results in a precocious induction of a subset of nerve injury-induced genes in the absence of c-Jun induction. These results suggest that injury-associated genes are normally repressed by H3K27 trimethylation, and that removal of H3K27 trimethylation is required for part of the injury response program in Schwann cells.

S09-03

Control of myelinating cell plasticity by chromatin-remodeling enzymes

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While the peripheral nervous system (PNS) can fully regenerate after a lesion, regeneration in the central nervous system (CNS) is very limited. This is largely due to the presence of different types of glial cells in these two systems. Schwann cells, the myelinating glia of the PNS, can support axonal regrowth, whereas oligodendrocytes, the myelinating glia of the CNS, cannot. The regenerative properties of Schwann cells result from their remarkable plasticity that enables them to dedifferentiate and redifferentiate after a nerve lesion, and thereby foster axonal regrowth and rebuild myelin.

Our aim is to identify the main mechanisms that explain the different plasticity of Schwann cells and oligodendrocytes after lesion. Because many genes are strongly regulated in Schwann cells after a PNS lesion, our lab is interested in the potential involvement of chromatin remodeling in the control of these genes. Local changes of chromatin architecture can be achieved by post-translational modifications of histones such as methylation, acetylation, phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation. These changes are dynamic and allow for rapid repression or de-repression of target genes. In addition, chromatin remodeling enzymes such as histone acetyltransferases and histone deacetylases can respectively acetylate and deacetylate transcription factors and thereby modify their activity. These enzymes are therefore very potent transcriptional regulators.

We find that several chromatin remodeling enzymes are strongly regulated in Schwann cells after a sciatic nerve lesion, whereas no regulation of their expression occurs in oligodendrocytes after a spinal cord lesion. Consistent with their robust upregulation in Schwann cells, we identify their critical functions in the regulation of key genes of the regeneration process. During this symposium, I will present and discuss our latest findings on this topic.

S09-04

The role of class II HDACs in the transition towards the Schwann cell repair phenotype

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After nerve injury, myelinating Schwann cells trans-differentiate into a specialized repair-promoting phenotype (the Bungner cell) that stimulates axon growth and directs them back towards the target tissues. This process is mediated by the AP-1 transcription factor c-Jun. Our results suggest that a drop in the cAMP levels in the injured nerve activates the expression of this transcription factor and the trans-differentiation program. Our data also suggests that the effects of cAMP are mediated by the translocation of histone deacetylase 4 (HDAC4) into the nucleus of Schwann cells. Gain of function experiments (using the mutant HDAC4 3SA GFP) demonstrate that this translocation is enough to block c-Jun expression and to switch on the differentiation program in Schwann cells. We also show that the effect of HDAC4 on c-Jun expression is mediated by its capacity to recruit the deacetylase activity of HDAC3 through the NcoR1-SMRT family of proteins.

SYMPOSIUM 10
Developmental signaling mechanisms in gliogenesis

S10-01

A role for TACE/ADAM17 during oligodendrocyte development and regeneration

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Several studies have elucidated the significance of a disintegrin and metalloproteinase proteins (ADAMs) in PNS myelination, but there is no evidence whether they also play a role in oligodendrogenesis and CNS myelination. Our study identifies ADAM17, also called tumor necrosis factor-α converting enzyme (TACE), as an essential modulator of oligodendrocyte development and regeneration following demyelination. By using genetic mouse models to achieve selective gain- or loss-of-function of TACE or EGFR in oligodendrocyte lineage cells *in vivo*, we found that TACE is critical for EGFR activation in oligodendroglial cells, and therefore, for sustaining oligodendrocyte development, during postnatal CNS myelination and during remyelination following Cuprizone-induced demyelination. TACE deficiency in oligodendrocyte progenitor cells compromises cell expansion, and oligodendrocyte differentiation and survival, delaying the process of CNS myelination and remyelination. Mechanistically, TACE modulates the shedding of the EGFR ligands TGFα and HB-EGF and, consequently, EGFR signaling activation in OL lineage cells during CNS myelination and remyelination. EGFR overexpression in TACE deficient oligodendrocytes *in vivo* restores oligodendrocyte development and postnatal CNS myelination, but also oligodendrocyte regeneration and CNS remyelination following demyelination. Our study reveals an essential function of TACE in supporting oligodendrocyte development during CNS myelination and remyelination that may contribute to the design of new strategies for therapeutic intervention in demyelinating disorders by promoting oligodendrocyte regeneration and myelin repair.

S10-02

The role of RAS-ERK signaling in specifying glial cell fates in neocortical development and gliomagenesis

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Neural cell fate specification is well understood in the embryonic cerebral cortex, where the proneural genes *Neurog2* and *Ascl1* are key cell fate determinants. What is less well understood is how cellular diversity is generated in brain tumors. Gliomas and glioneuronal tumours, which are often localized in the cerebrum, are both characterized by a neoplastic glial component, but glioneuronal tumours also have an intermixed neuronal component. A core abnormality in both tumor groups is overactive RAS/ERK signaling, a pro-proliferative signal whose contributions to cell differentiation in oncogenesis are largely unexplored. We found that RAS/ERK activation levels differ in two distinct human tumours associated with constitutively active BRAF. Pilocytic astrocytomas, which contain abnormal glial cells, have higher ERK activation levels than gangliogliomas, which contain abnormal neuronal and glial cells. Using *in vivo* gain- and loss-of-function in the mouse embryonic neocortex, we found that RAS/ERK signals control a proneural genetic switch, inhibiting *Neurog2* expression while inducing *Ascl1,* a competing lineage determinant. Furthermore, we found that RAS/ERK levels control *Ascl1's* fate specification properties in murine cortical progenitors - at higher RAS/ERK levels, *Ascl1*$^{+}$ progenitors are biased towards proliferative glial programs, initiating astrocytomas, while at moderate RAS/ERK levels, *Ascl1* promotes GABAergic neuronal and less glial differentiation, generating glioneuronal tumors. Mechanistically, Ascl1 is phosphorylated by ERK, and ERK phosphoacceptor sites are necessary for *Ascl1's* GABAergic neuronal and gliogenic potential. RAS/ERK signaling thus acts as a rheostat to influence neural cell fate selection in both normal cortical development and gliomagenesis, controlling *Neurog2-Ascl1* expression and *Ascl1* function.

S10-03

The endocannabinoid system controls radial glia transition to intermediate progenitor cells via mTORC1 signalling

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The endocannabinoid system via the CB_1 cannabinoid receptor regulates different aspects of cortical development. CB_1 receptor signalling engaged by the local production of the endocannabinoids 2-arachidonoylglycerol and anandamide controls neural precursor cell proliferation, neuronal differentiation and axonal connectivity. In this presentation the contribution of CB_1 receptor-signaling to radial glial cell cycle maintenance and transition to intermediate progenitor cells will be discussed. CB_1-deficient mouse embryos show premature cell cycle exit, decreased Pax6- and Tbr2-positive cell number, and reduced mammalian target of rapamycin complex 1 (mTORC1) activation in the ventricular and subventricular zones. Pharmacological stimulation of the CB_1 receptor in cortical slices and progenitor cell cultures activated the mTORC1 pathway and increased the number of Pax6- and Tbr2-expressing cells. Likewise, acute CB_1 knockdown *in utero* reduced mTORC1 activation and cannabinoid-induced Tbr2-positive cell generation. Luciferase reporter and ChIP assays revealed that the CB_1 receptor drives Tbr2 expression downstream of Pax6 induction in an mTORC1-dependent manner. Altogether, our findings demonstrate that the CB_1 receptor tunes dorsal telencephalic progenitor proliferation by sustaining the transcriptional activity of the Pax6/Tbr2 axis via the mTORC1 pathway. The involvement of altered CB_1 receptor signaling in developmental disorders associated to aberrant neural precursor biology will be discussed.

S10-04

From expanding to reprogramming NG2 glia

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Here I will discuss our efforts to understand the regulation of oligodendrogliogenesis in the adult subependymal zone using single cell tracking as well our attempts of lineage reprogramming NG2 glia following injury into induced neurons.

In the first part, I will show our evidence that within the adult niche of the subependymal zone Wnt signaling acts specifically on the oligodendroglial lineage by enhancing progenitor proliferation. Our data support a model according to which neurogenic and oligodendrogliogenic neural stem cells constitute strictly separate lineages.

In the second part, I will show data demonstrating that NG2 glia of the cerebral cortex in vivo can be lineage reprogrammed into doublecortin-positive immature neurons by forced expression of the transcription factor Sox2. Intriguingly, this lineage conversion was only observed upon injury, suggesting that lesion-induced changes in NG2 glia and/or their environment are critical for reprogramming to occur.

SYMPOSIUM 11
Neurons and microglia: bidirectional communication in health and diseases

S11-01

Microglial regulation of synaptic function

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Under physiological conditions, microglia are present in all regions of the adult brain at rather high density. Microglial cells are highly dynamic and react rapidly to the modifications of their environment. They express membrane receptors for all known neurotransmitters and are thus putatively able to sense neuronal activity and/or to communicate with astrocytes. Therefore, microglia display functional features of synaptic partners, but their involvement in the regulation of synaptic transmission has often been overlooked. We previously showed that activation of microglia induces a rapid production of ATP that induces glutamate release from astrocytes. The astrocytic glutamate then increase the EPSC frequency through neuronal mGluR5. We recently explored how microglia also regulate inhibitory neurotransmission. We demonstrated that microglia achieve a tonic but also a rapid control on inhibitory synaptic strength by controlling the dynamic of inhibitory neurotransmitters at synapses. These results reveal microglia as a genuine regulator of both excitatory and inhibitory neurotransmission.

S11-02

Neuronal hyperactivity triggers microglial phagocytosis-apoptosis uncoupling

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Phagocytosis is an essential component of the brain regenerative response, and is assumed to be executed by ameboid microglia. In contrast, we herein show that phagocytosis by surveillant, ramified microglia is efficiently coupled to apoptosis during physiological conditions, excitotoxicity, and inflammation in the hippocampus in vivo and in vitro. Unexpectedly, in human and mouse mesial temporal lobe epilepsy, a major neurological disorder characterized by excitotoxicity and inflammation, microglial phagocytosis is impaired early on. This phagocytic blockade is not directly mediated by glutamate receptors on microglia, but rather by the hyperactivity of the hippocampal network, which disrupts local ATP microgradients and prevents the efficient targeting of apoptotic cells. Ultimately, microglial phagocytosis impairment leads to the accumulation of non-phagocytosed apoptotic cells and the development of an inflammatory response. These results suggest that the decreased efficiency of microglial phagocytosis contributes to the early pathophysiology of epilepsy and possibly other neurodegenerative and neurological disorders characterized by neuronal death and inflammation.

S11-03

In vivo studies of microglial function in synapse formation

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Microglia are the resident macrophages of the central nervous system and their functions have been extensively studied in various brain pathologies. The physiological roles of microglia in brain plasticity and function remain unclear. We have generated CX_3CR1^{CreER} mice expressing tamoxifen-inducible Cre recombinase that allow for specific manipulation of gene function in microglia. Using CX_3CR1^{CreER} to drive diphtheria toxin receptor expression in microglia, we found that microglia could be specifically

depleted from the brain upon diphtheria toxin administration. Mice depleted of microglia show deficits in multiple learning tasks and a significant reduction in motor learning-dependent synapse formation. Furthermore, Cre-dependent removal of brain-derived neurotrophic factor (BDNF) from microglia largely recapitulated the effects of microglia depletion. These results reveal important physiological functions of microglia in learning and memory by promoting learning-related synapse formation through BDNF signaling. We are currently investigating the role of microglia and microglial BDNF signaling in regulating synaptic plasticity in mouse models of chronic pain and traumatic brain injury.

S11-04

Structural interactions between microglia and synapses in contexts of neuroinflammation

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This past decade has witnessed a revolution in our understanding of microglia, especially since their roles in the healthy brain have started to unravel. These cells were shown to actively maintain health, in concert with neurons and other types of glial cells, providing further insight into their crucial involvement with diseases.

To set microglia on the stage, I will begin my talk by explaining briefly who they are, what they do in health and disease, and how they can be studied using non-invasive imaging approaches. I will also introduce microglia-synapse interactions and their emerging roles in the experience-dependent remodeling of neuronal circuits during normal physiological conditions.

The body of my talk will be dedicated to the significance of these new roles in the healthy brain and one underlying molecular mechanism: signalling between the chemokine fractalkine (CX3CL1) mainly expressed by neurons and its unique receptor CX3CR1 exclusively found on microglia. In particular, I will present our recent work showing that this pathway regulates microglial phenotype, microglia-synapse interactions, synaptic plasticity and function, and the behavioural outcome following chronic unpredictable stress, an environmental challenge associated with a massive remodeling of neuronal circuits and elimination of synapses.

Lastly, I will present a possibly new microglial phenotype: the mysterious “dark” cells, which extensively interact with synapses in various contexts of health and disease.

SYMPOSIUM 12
Immunity and remyelination

S12-01

Ageing macrophages and failing remyelination

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Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent stem cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in multiple sclerosis (MS), and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in MS, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. There is now compelling evidence that 1) the innate immune system plays a key beneficial role in remyelination and 2) ageing is the major contributor to the declining efficiency of remyelination and that this is largely due to age-associated changes in the innate immune response to demyelination. This talk will review

recent studies we have undertaken aimed at obtaining a detailed understanding of the effects of age on macrophages during remyelination and hence identifying novel therapeutic targets.

S12-02

Macrophage activation in perinatal white matter injury

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Cerebral palsy is a lifelong disorder characterized by severe motor deficits, and is incurred perinatally by inflammation and hypoxia in the brain. This induces proliferation of oligodendrocyte progenitors yet prevents oligodendrocyte precursor cell survival and differentiation into myelinating oligodendrocytes, causing hypomyelination and impaired nerve function. Current treatments aiming to dampen initial brain injury or manage symptoms are only marginally effective, highlighting the need for therapies that promote myelin repair. Our previous work showed that during efficient myelin repair in the adult brain, microglia and circulation-derived macrophages undergo a switch in activation from a pro-inflammatory phenotype which drives oligodendrocyte progenitor proliferation, to a regenerative phenotype which drives oligodendrocyte precursor survival/ differentiation and myelin repair. We hypothesized that following perinatal brain injury, pro-inflammatory microglia/ macrophage phenotypes predominate over regenerative phenotypes to drive the abovementioned pathology. Our analysis of post-mortem human brain tissue of perinatal brain injury and *in vivo* experimental models of this injury demonstrated an imbalance of microglia/ macrophage activation towards the pro-inflammatory phenotype. We developed a novel *ex vivo* forebrain explant model of perinatal brain injury induced by LPS-mediated sensitization followed by hypoxia, characterized by oligodendrocyte progenitor proliferation, precursor cell death, lack of differentiation and myelination. This was associated with a high ratio of microglia with a pro-inflammatory vs regenerative phenotype. Oligodendrocyte differentiation and/or survival were rescued by supplementation with conditioned media from regenerative microglia or novel factors we identified to be secreted by these cells. Our findings demonstrate that perinatal brain injury modulates microglia/ macrophage activation towards a pro-inflammatory phenotype unable to support oligodendrocyte precursor differentiation and survival, which can be rescued by factors secreted from microglia with a regenerative phenotype. This work has identified microglia and their secreted products as novel therapeutic targets to drive myelin repair following perinatal brain injury.

S12-03

Regulatory T cells directly promote myelin regeneration in the Central Nervous System

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Enhancement of myelin regeneration (remyelination) in the Central Nervous System (CNS) is an unmet therapeutic need. Successful remyelination requires adult CNS progenitors to differentiate into myelin-producing oligodendrocytes[1]. In Multiple Sclerosis (MS), this differentiation step is a key bottleneck causing remyelination failure[2]. Given that inflammation-resolving effects of regulatory T cells (Treg) coincide with tissue regeneration, we hypothesized that Treg promote remyelination in the CNS. In our studies, Treg depletion impaired oligodendrocyte differentiation following spinal cord demyelination *in vivo*. Treg or Treg-conditioned media enhanced oligodendrocyte differentiation in organotypic brain slices, mixed glia and purified oligodendrocyte progenitor cell cultures, as well as accelerating (re)myelination of neuron-glia cultures *in vitro* and brain slices *ex vivo*. Our data reveal a direct effect of Treg on CNS progenitor cells and that Treg directly boost myelin regeneration in the CNS. Harnessing this regenerative Treg effect holds potential to develop new therapies for demyelinating diseases.

S12-04

Enhancement of the activity of M2-polarized macrophages/microglia promotes recovery from demyelination

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Macrophages and CNS-resident microglia are broadly classified into pro-inflammatory M1 and anti-inflammatory M2 subtypes, and they may switch from one state to the other depending on the microenvironment. Using well-described culture conditions, specifically interferon-γ/lipopolysaccharide (LPS) and IL-4/IL-13 respectively, we have generated M1 and M2 cells in order to address their effects on neurons and oligodendrocyte precursor cells (OPCs) in culture, on altering the functions of the chondroitin sulfate proteoglycans (CSPGs) implicated in inhibiting remyelination, and on mediating recovery from demyelination in mice. Unexpectedly, we discovered that the LPS stimulation of M2 cells during their polarization further enhanced M2 characteristics and their beneficial properties, in part through elevation of IL-4 receptor and IL-10 expression. The LPS-enhanced M2 cells did not kill neurons and OPCs in culture, unlike the M1 cells generated by interferon-γ and LPS. To investigate the impact of polarized macrophages/microglia in vivo, we inflicted demyelination of the dorsal column of the mouse spinal cord using lysolecithin; 3 days after, saline, M1 and M2-polarizing conditions, or M2+LPS were locally applied at the site of injury. Treatment that generated M1 exacerbated injury, M2 had negligible effect on the evolving insult, while the LPS-enhanced M2 treatment diminished the accumulation of inhibitory CSPGs, increased the number of oligodendrocyte precursors, and reduced lesion volume. Ongoing experiments are aimed at defining the lesion microenvironment resulting from the LPS-enhanced M2 treatment, and extent of remyelination. These data suggest that toll-like receptor-4 stimulation of M2 cells during their polarization can dramatically enhance their beneficial M2 characteristics to favor recovery from CNS insults.

SYMPOSIUM 13
Paradigm shift in astrocyte calcium signalling

S13-01

Macroscopic and microscopic calcium dynamics of astrocytes

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Astrocytes spontaneously exhibit intracellular calcium elevations in various brain regions both *in vitro* and *in vivo.* In this symposium, we will talk about two topics, (1) spontaneous calcium oscillations in the somata in vitro and (2) spontaneous spot-like activity in astrocytic processes in vivo.

First, we utilized a long-term optical imaging technique and analyzed calcium activities in a total of more than 4,000 astrocytes in mouse hippocampal slices. Astrocytic calcium activity was largely sparse and irregular, but we found a subset of cells exhibiting calcium oscillations during which a few calcium fluctuations repeated at a regular interval of approximately 30 s. The calcium oscillations i) consisted of a complex reverberatory interaction between the soma and processes within astrocytes, ii) did not synchronize with those of other astrocytes or require neuronal firing, iii) depended on type 2 inositol 1,4,5-trisphosphate receptors and were modulated by cAMP-protein kinase A signaling, and iv) were facilitated under pathological conditions such as energy deprivation and epileptiform hyperexcitation and were associated with enhanced hypertrophy in astrocytic processes, an early hallmark of reactive gliosis found in ischemia and epilepsy. Therefore, calcium oscillations may be linked to a pathological state in astrocytes.

In the second part, we visualized the calcium signals in astrocytes in the primary visual cortex of awake, head-fixed mice. Two-photon imaging revealed sparse responses by astrocytic processes to visual stimuli with variable spot-like patterns of calcium elevations. The subcellular calcium domains

that were activated by visual stimuli were larger in size but not more frequent than spontaneously occurring domain activity, and they exhibited no apparent orientation tuning. The evoked calcium domains were distributed unevenly within the cell, constituting multiple active “hotspots” that were often also recruited in spontaneous activity. Thus, the patterns of astrocytic calcium dynamics are intrinsically constrained and are subject to minor modulation by sensory input.

S13-02

Spatiotemporal calcium dynamics in single astrocytes and its modulation by neuronal activity

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Calcium dynamics underlies various functions of astrocytes such as regulation of synaptic plasticity and local blood supply. For a long time general belief was that information is coded in the frequency of astrocytic calcium events which changes in response to neuronal or neighboring astrocytes activity. For example synaptic release of neurotransmitters thought to trigger new calcium events in perisynaptic astrocytic processes (PAPs) though activation of metabotropic glutamate receptors (mGluRs). Our recent findings suggest that PAPs are devoid of calcium stores that are required for this kind of calcium signaling. This makes unlikely any significant role of mGluRs in triggering calcium events in PAPs. Instead we show that activation of ‘extrasynaptic’ astrocytic mGluRs increases proportion of spatially extended calcium events in the power-law based distribution of calcium event sizes. This effect takes place without any significant increase in the frequency of calcium events. This suggests that astrocytic response to surrounding brain activity is coded in spatial characteristics of their calcium events, which is fundamentally different from temporal information coding in neurons (e.g. coincidence detection, neuronal acting potentials sequences etc). Our analysis of whole calcium events in the series of astrocytic florescence images also explains how previous reports could get false increases in the events frequency due to activation of mGluRs using region-of-interest based analysis.

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S13-03

Fundamental differences between spontaneous vs. low-level evoked astrocyte calcium activity

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Astrocyte Gq GPCR and IP_3 receptor-dependent Ca^{2+} elevations occur spontaneously *in situ* and *in vivo*. These events vary considerably in size, often remaining confined to small territories of astrocyte processes called “microdomains” and sometimes propagating over longer distances that can include the soma. It has remained unclear whether these events are driven by constitutive (basal) GPCR signaling activity, neuronal action potential-dependent or quantal vesicular release, or some combination of these mechanisms. Here, we applied manipulations to increase or inhibit neuronal vesicular neurotransmitter release together with low-level stimulation of Schaffer collaterals in acute mouse hippocampal slices in an effort to determine the mechanisms underlying spontaneous astrocyte Ca^{2+} events. We found no significant change in spontaneous microdomain astrocyte Ca^{2+} elevations when neuronal action potentials were significantly enhanced or blocked. The astrocyte Ca^{2+} activity was also not affected by inhibitors of Group I mGluRs. However, blockade of miniature neurotransmitter release using Bafilomycin A1 significantly reduced the frequency of microdomain astrocyte Ca^{2+} elevations. We then tested whether astrocyte Ca^{2+} microdomains can be evoked by low intensity SC stimulation. Importantly, microdomains could not be reproduced even using single, low intensity pulses to the SCs at a minimum distance from the astrocyte. Evoked astrocyte Ca^{2+} responses most often included the cell soma, were reduced by group I mGluR antagonists, and were larger in size compared to spontaneous Ca^{2+} microdomains. Overall, our findings suggest that spontaneous microdomain astrocyte Ca^{2+} elevations are not driven by neuronal action potentials but

require quantal release of neurotransmitter which cannot be replicated by stimulation of Schaffer collaterals.

S13-04

Glial calcium dysregulation in Alzheimer's disease

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Alzheimer's disease is neurodegenerative disorder that as synaptic dysfunction and neuronal death as the central features that lead to the clinical features. The most accredited hypothesis of the pathogenesis sees accumulation of beta-amyloid protein in the brain (in both soluble and insoluble forms) as a leading mechanism of neurotoxicity. How b-amyloid triggers the neurodegenerative disorder is at present unclear, and up to recently the main view was that the beta-amyloid protein, synthesised by neurons, led to neurodysfunction and loss of neurons. Furthermore, growing evidence suggests that a deregulation of Ca^{2+} homeostasis and deficient Ca^{2+} signalling may represent a fundamental pathogenic factor.

Recently, our lab, among others has reviseted the Ca20+-hypothesis of alzheimer by investigting whether similar changes to those described in neurons also occur in astroglial cells. Indeed, exposure of cultured neurons to nanomolar, pathologically relevant, concentrations of beta amyloid appear to finely dys-regulate basal callcium levels in glial cells, and this in turn activates an abherrant gene transcription program mediated, at least in part, by NFAT and NFkB. The talk will also review data from in vivo and human studies to strenghen the conclusion that deregulation of Ca^{2+} homeostasis and signalling in astrocytes is likely to contribute to the pathogenenesis of Alzheimer's disease.

SYMPOSIUM 14
The role of glial cells in peripheral neuropathy

S14-01

Regulation of Schwann cell physiology by LRP1: role in neuroinflammation, regeneration and neuropathic pain

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Chronic neuropathic pain is a debilitating disorder that affects millions of Americans. Physical trauma to peripheral nerves and non-functional nerve regeneration are important causes of neuropathic pain. Infiltration of the affected nerves by inflammatory cells plays a major role. Therapeutic options for painful peripheral neuropathy are limited. New drugs that target the molecular pathways that cause chronic pain are needed. Schwann cells (SCs) play a major role in the response to PNS injury and may represent an overlooked and promising new target for the development of more effective treatments for chronic neuropathic pain. We have identified LDL Receptor-related Protein-1 (LRP1) as a major cell-signaling receptor in SCs. LRP1 promotes SC survival and migration and regulates levels of pro-inflammatory cytokines and chemokines. When LRP1 is conditionally deleted in SCs in mice, demyelination proceeds more rapidly, neuro-inflammation is greatly increased, and pain-related behaviors are exacerbated after injury. In wild-type mice, SC LRP1 signaling may be augmented by therapeutic delivery of specific LRP1 ligands to injured nerves. We have characterized receptors, such as N-methyl-D-aspartate (NMDA) receptors, that function with SC LRP1 as a biochemical system to induce the changes in SC physiology observed after PNS injury. Moreover, augmentation of LRP1 signaling alters the course of PNS injury, attenuating neuro-inflammation and preserving axons. We take advantage of recombinant proteins engineered in our laboratories to activate LRP1 signaling without off-target effects. Our results suggest that SC LRP1 alters key regulatory components of Schwann cell-axon interactions, which is most evident after injury. Mice in which LRP1 is deficient in

SCs represent a model for studying how abnormalities in Schwann cell physiology may facilitate and sustain chronic pain.

S14-02

Treating small fiber neuropathy by topical application of a small molecule GFRα/RET modulator

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Small fiber neuropathy is a disorder of peripheral nerves commonly found in patients with diabetes mellitus, HIV infection, and those receiving chemotherapy. The complexity of disease etiology has led to a scarcity of effective treatments. Using two models of progressive small fiber neuropathy, we show that over-expression of glial cell line-derived neurotrophic factor (GDNF) in skin keratinocytes or topical application of XIB4035, a reported non-peptidyl agonist of GDNF receptor alpha-1 (GFRα1), are effective treatments for small fiber neuropathy. We also demonstrate that XIB4035 is not a GFRα1 agonist, but rather it enhances GFRα family receptor signaling in conjunction with ligand stimulation. Taken together, our results indicate that topical application of GFRα/RET receptor signaling modulators may be a novel therapy for small fiber neuropathy, and we have identified XIB4035 as a candidate therapeutic agent.

S14-03

Challenges in developing novel therapies for peripheral neuropathies: Targeting Glia

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In the peripheral nervous system (PNS), Schwann cells play a key role in maintenance of long peripheral axons and in the pathology of various inflammatory, metabolic and hereditary polyneuropathies. As the major glial cells of the PNS, Schwann cells often play a neuroprotective role and help prevent distal axonal degeneration against various insults. However, dysfunction in Schwann cells may also indirectly lead to distal axonal degeneration, hallmark of most peripheral neuropathies. For example, in human immunodeficiency virus (HIV) - associated sensory neuropathy, perineuronal Schwann cells in the dorsal root ganglia indirectly result in activation of caspase pathway in sensory neurons by the viral envelop proteins, but the peri-axonal Schwann cells provide a neuroprotective support. In many inherited neuropathies caused by mutations in genes involved in myelin formation and maintenance, Schwann cell dysfunction and chronic demyelination leads to axonal degeneration. In metabolic conditions such as diabetes, there is clear evidence of Schwann cell dysfunction that exacerbates the metabolic strain on axons that cause degeneration. Understanding the molecular underpinnings of axon-Schwann cell interactions using in vitro and in vivo models of peripheral neuropathies may help identify novel targets for drug development for peripheral neuropathies.

S14-04

Schwann cell reactions and mutual interactions with macrophages in myelin mutants: implications for treatment of inherited neuropathies

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Previous studies from our laboratory have shown that Schwann cells of connexin 32-deficient mice (Cx32def), a model for the X-linked form of Charcot-Marie-Tooth neuropathy (CMT1X), show

upregulation of c-Jun and GDNF, which likely mediate or facilitate the typical axonal sprouting in these mutants. These Schwann cell reactions are reminiscent of regeneration patterns in Wallerian degeneration (WD) and might, therefore, serve as chances to treat the disorders in the future by fostering this intrinsic repair mechanism.

Another WD-like reaction of the mutant Schwann cells is the increased activation of MEK-ERK signalling that causes upregulation of monocyte chemoattractant protein-1 (MCP-1; Ccl2) and mediates recruitment and activation of pathogenic macrophages in this model. Colony-stimulating factor-1 (Csf-1) is another important cytokine for activation of nerve-damaging macrophages and is - as opposed to MCP-1/Ccl2 - expressed by endoneurial fibroblasts as a reaction upon a still unknown signal likely originating from the mutant Schwann cells. Interestingly, the Csf-1-dependent macrophages in peripheral nerves of the CMT1X model are not only unspecific phagocytes for mutant myelin, but are target-directed (possibly by MCP-1/Ccl2 and nerve fiber-accumulating antibodies) and essential mediators of disease-related Schwann cell dedifferentiation and dysfunction.

Based on these findings, we considered the possibility that pathogenic, macrophage-related peripheral nerve inflammation could be a putative target to ameliorate disabling symptoms associated with CMT1X. As an approach closely related to clinical translation, we orally treated the CMT1X models with a novel and highly selective Csf-1-receptor (Fms kinase) inhibitor (provided by Plexxikon Inc.), which was tested in a phase 1 clinical trial for the treatment of rheumatoid arthritis. By this approach, we could substantially improve peripheral nerve pathology and function.

Our experiments demonstrate that inherited peripheral neuropathies share some molecular and cellular features with WD that could either serve as treatment chances (e.g., intrinsic axonal sprouting) or as therapeutic targets (e.g., macrophages causing disease-related Schwann cell dedifferentiation and dysfunction) in inherited neuropathies and possibly other disorders of the peripheral nervous system.

SYMPOSIUM 15
Epigenetics shapes glial functions in health and disease

S15-01

Epigenetic control of microglia polarization in brain pathologies

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Microglia polarization refers to establishment of a specific phenotype important for tissue homeostasis or host defense in response to specific cues. In diseased brain pathological factors induce microglia polarization which results in production of cytokines, chemokines and growth factors shaping brain microenvironment and the immune system response. The inflammatory (M1) and alternative, anti-inflammatory (M2) phenotype are the extreme states of microglial activation that can produce detrimental or beneficial effects. It is poorly understood how microglia are reprogrammed to alter their responses to environmental challenges and how signals are converted into sustained patterns of gene expression. We studied contribution of epigenetic mechanisms to modulating M1/M2 polarization of microglia. Transcriptome analysis of primary microglial cultures exposed to glioma-conditioned medium (GCM) or lipopolysaccharide (LPS) revealed activation of distinct signaling and metabolic pathways resulting in different patterns of gene expression. DNA methylation analysis reveals the lack of DNA methylation at the promoters of genes crucial for either phenotype. Chromatin immunoprecipitation with four antibodies that recognize common activating and repressive histone modifications revealed the decrease of histone acetylation in microglia exposed to GCM for 6 h due to the increased histone deacetylase (HDAC) activity. Changes in repressive histone modifications after GCM or LPS were delayed and correlated to transcription down-regulation that suggests their role in consolidation of the phenotype towards which the cell is polarized. HDAC inhibitors blocked morphological changes associated with GCM or LPS treatment and decreased the expression of GCM up-regulated genes to the level observed in control microglia. Furthermore, HDAC inhibitors restored

the expression of LPS-inducible genes inhibited by GCM pretreatment that suggests a possibility of reprogramming of gene expression. These results demonstrate that the inflammatory genes are epigenetically "primed" and easy to be induced in microglia, while the erasure of histone acetylation marks is prerequisite to put the repressive epigenetic marks on M1 inflammatory genes and induce acetylation of M2 genes are crucial for glioma-induced microglia polarization. Inhibition of histone modifying enzymes blocks acquisition of a specific phenotype.

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S15-02

Epigenetic regulation of the microglia inflammatory response

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Microglia, the innate immune cells of the central nervous system (CNS), react to endotoxins like bacterial lipopolysaccharides (LPS) with a pronounced inflammatory response. To avoid excess damage to the CNS, the microglia inflammatory response needs to be tightly regulated. A single LPS challenge results in a prolonged blunted pro-inflammatory response to a subsequent LPS stimulation, both in primary microglia cultures and *in vivo* in mice. Chromatin immunoprecipitation (ChIP) experiments revealed that LPS preconditioning was accompanied by a reduction in active histone modifications AcH3 and H3K4me3 and an increase in repressive histone modification H3K9me2 in inflammatory promoters. ChIP and knock-down experiments showed that NF-κB subunit RelB is required for the attenuated LPS response. In addition to a suppressed pro-inflammatory response, preconditioned primary microglia displayed enhanced phagocytic activity, increased outward potassium currents and nitric oxide production in response to a second LPS challenge. Summarizing, LPS-preconditioned microglia acquire an epigenetically regulated, immune-suppressed phenotype, possibly to prevent excessive damage to the central nervous system in case of recurrent (peripheral) inflammation.

S15-03

Microglia and the developing brain: implications for long-term neural function

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The study of neural-glial interactions within the brain is in its infancy. However, there is mounting evidence that microglia and astrocytes, the primary immunocompetent cells of the CNS, are involved in every major aspect of brain development and function, including neurogenesis and migration, synaptic formation and pruning, and phagocytosis of apoptotic debris. Many cytokines and chemokines (e.g., interleukin [IL]-1β and CCL2, respectively) are produced by glia at much higher baseline levels in the developing brain compared to the adult brain, although this time course depends highly on brain region and sex. These collective data have led us to hypothesize that early development represents a sensitive period for immune perturbation and thus long-term alterations in neural function, because the immune system is so active within the brain at this time. Indeed, evidence from both animal and human studies implicates the immune system in a number of disorders with known or suspected developmental origins. This talk will focus on the evidence that diverse challenges (e.g., infection, toxins, drugs of abuse) during distinct stages of development act as a vulnerability factor for later-life alterations in central immune signaling, and marked changes in behavior throughout the remainder of the lifespan, with a focus on the hypothesis that epigenetic changes in microglial cell function may underlie such vulnerability.

S15-04

Microglia phagocytic activity and autism

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Tissue homeostasis is achieved when a state of equilibrium is reached between two major processes: clearance of dying cells and generation of new ones. Numerous diseases are accompanied by an increase in death processes and a decrease in regenerative capacity, resulting in deterioration and malfunction of the tissue. Dead cells must be cleared to make room for new cells and to remove toxic compounds that the dying cells produce.

The effect of impaired phagocytic activity of brain professional phagocytes (microglia) and other tissue resident macrophages will be discussed here in the context of Rett syndrome and its potential implication to a wide variety of animal models for autism. We propose the microglia, in general, and phagocytic activity, in particular, as new targets for future therapeutic intervention for autism spectrum disorders.

SYMPOSIUM 16
Role of microglial cells in the control of T-cell responses within the CNS

S16-01

Role of microglia, macrophages and dendritic cells in the antigen presenting mechanisms associated to EAE

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Lymphocytic activation involves three main signals, the first provided by the engagement of major histocompatibility complex molecules (MHC) expressed in the surface of antigen presenting cells (APCs) with the receptor of T-cells (TCR), the second by the binding of specific co-stimulatory molecules in the membrane of both APC and T-cells, and the third by the secretion or expression of T-cell polarising molecules in specific populations of APCs. Microglial cells are considered to be the main APC population in the central nervous system (CNS) because they are able to provide the three signals under specific situations. However, a growing number of evidences suggest that professional dendritic cells (DCs), the main APC population in the peripheral immune system, may also participate in the regulation of T-cell responses taking place within the CNS. The origin of DCs in the CNS is still not well known but in addition to their possible recruitment and infiltration from the periphery, one of the theories points towards a putative microglial origin.

Experimental autoimmune encephalomyelitis (EAE), a well-established model of multiple sclerosis, is characterised by microglial activation and lymphocytic infiltration. Specifically in the acute model of EAE induced in Lewis rat, we have reported an increase in MHCs, co-stimulatory molecules and expression of different T-cell polarising factors in activated microglial cells along the different phases of the disease. These observations points towards microglial cells as the principal regulators of T-cell responses. However, in addition to microglia, we have also found a specific population of perivascular DCs (ED2+ cells and fascin+ cells) which are able to migrate into the CNS parenchyma, specifically during the peak of EAE, suggesting that these cells may also play a role in lymphocytic regulation.

In this presentation we will discuss the current knowledge regarding microglial/macrophage function in EAE, their role in T-cell modulation and the participation of professional DCs in the immune response associated to this disease.

S16-02

Microglia as regulators of T cell response in the CNS

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Microglia are the tissue macrophages of the central nervous system (CNS). Their primary functions are as part of innate CNS immunity, to clear debris and damaged cells, and to signal for lymphocyte response in adaptive immunity. Microglia function as antigen presenting cells (APC) for T cell proliferative response, and although they may be outperformed in this respect by infiltrating APC's in the context of autoimmune neuroinflammation, subsets such as $CD11c^{+}$ microglia are very effective APC for proliferative response while differing in the nature of the response induced. Microglia are increasingly seen to play a major role in homeostatic regulation in the CNS. Microglia modulate T cell and other immune responses in the CNS via secretion of selective profiles of cytokines that influence the local immune environment, such as Type I interferons, as well as chemokines. Cytokine secretion profiles and microglial phenotypes are themselves influenced by developmental and degenerative cues, including bidirectional interaction with astrocytes, and their recent phagocytic experience. They therefore interpret local needs and conditions for appropriate immune response. Understanding how these regulatory profiles are triggered and maintained will be key to therapeutic exploitation

S16-03

Fighting Alzheimer's disease and brain aging neuroinflammation via the brain's choroid plexus

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Over the past decades, it became clear from our studies that the immune system has pivotal roles in central nervous system (CNS) function, including neurogenesis, cognitive capacity, maintenance and repair, and the ability to cope with stressful conditions and to fight off neurodegenerative diseases. However, since interactions of neurons or glial cells with T lymphocytes rarely occur within the healthy CNS parenchyma, the underlying mechanism of this protective response remained a mystery. The CNS is separated from the rest of the body by the blood-brain-barrier and by the blood-cerebrospinal-fluid barrier, including the choroid plexus (CP) and the meninges. We suggested that the CP acts as a sentinel for danger signals coming from the CNS parenchyma and can translate these signals, through its dialogue with the T cells that reside in it, into mechanisms that support the healthy brain, and facilitate recruitment of leukocytes to the parenchyma, for resolution of local inflammation, in cases of pathology. When the microglia respond to emerging risk factors within the brain, they can enter into a vicious inflammatory cycle, the resolution of which necessitates infiltration of circulating immune resolving cells, such as anti-inflammatory cells and regulatory T cells. We found that during aging and AD, the CP dysfunctions and does not allow entry of sufficient numbers of resolving immune cells. Overcoming this CP dysfunction, enables its activation to allow trafficking of immunoregulatory cells to the brain parenchyma, affecting the microglia and the overall brain milieu. Amenable to immunomodulation, this interface is a unique target for arresting age-related cognitive decline and AD by optimizing its proper function as a selective and educative gate for recruitment of inflammation-resolving cells to the CNS parenchyma.

S16-04

T cell targeted attack on astrocytes triggers progressive epilepsy dependent on CNS influx of blood-derived antigen-presenting cells

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Epilepsy therapeutics are ineffective in 30% of patients and are accompanied by multiple side effects. Current antiepileptic drugs target neurotransmission. Unresponsiveness to one drug is highly predictive of unresponsiveness to all others, suggesting a shared disease etiology, requiring therapies targeted against non-neuronal targets. Innate immunity signaling, including proinflammatory cytokine modulation of astrocytic function has been implicated in epiletogenesis. Far less is known about the role of adaptive immunity and T cells. To determine whether an antigen-driven response against a non-neuronal target is sufficient to initiate epilepsy, we generated a transgenic mouse line in which astrocytes expressed a non-functional antigen serving as a recognition tag. These transgenic mice

were bred to T cell receptor transgenic mice in which >95% of CD4+ T cells recognize the transgenic tag antigen. Unmanipulated double transgenic appeared normal, but developed visually-overt seizures by 21 days post-treatment with pertussis toxin. Immunohistochemistry revealed CD4+ T cells infiltration throughout the brain including perivascular, periventricular, and parenchymal regions. The T cell response was strongly Th1-biased while seizure induction correlated with with a large influx of professional antigen-presenting cells. Lastly, using qPCR and flow cytometric analysis, we found increased expression of GFAP and glutamate transporter GLAST (EAAT1) indicative of astrocyte reactivity. Somewhat surprisingly, GLT-1 expression was not altered. In sum, our data provide proof of principle findings that T cell responses targeted against astrocytic and not neuronal targets are sufficient to trigger epileptogenesis. In addition, our data suggest that microglia play neuroprotective functions limiting T cell activation that can be overcome by the influx of large numbers of blood derived antigen-presenting cells.

SYMPOSIUM 17
Transcriptional regulation of oligodendrogenesis during myelination and remyelination

S17-01

Myrf promotes oligodendrocyte maturation both through positive regulation of myelin genes and negative regulation of OPC genes via induction of key micro-RNAs

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The differentiation of oligodendrocyte progenitor cells (OPCs) into myelinating oligodendrocytes is tightly coordinated by the opposing expression positive regulators of differentiation such as Sox10 and Myrf as well as negative regulators such as Hes5, Sox5 and Sox6. In addition, oligodendrocyte development is mediated by the activity of several microRNAs that are induced during differentiation and serve to repress these negative regulators. We have previously demonstrated that Myrf acts to directly induce the expression of many myelin genes during oligodendrocyte differentiation. Here, we find that in addition to its direct positive regulation of myelin genes Myrf is also required for the induction of miR-219 and miR-338 in differentiating oligodendrocytes. This induction is direct, with Myrf binding enhancer regions proximal to the two miRNAs during oligodendrocyte differentiation and promoting transcription from these elements. In the absence of Myrf miR-219 and 338 fail to be expressed in the differentiating oligodendrocytes, leading to abberant maintained expression of the inhibitors of differentiation Hes5, Sox5 and Sox6. These results identify Myrf-mediated induction of microRNAs as a mechanism to couple the induction of myelin genes with the repression of negative regulators of differentiation, ensuring a well-coordinated differentiation process.

S17-02

Chd7 chromatin remodeler function in oligodendrogenesis during (re)myelination

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Oligodendrocytes are myelin-forming cells of the central nervous system wrapping axons and therefore permitting the saltatory conduction of action potentials. Multiple sclerosis (MS) is a multifactorial disease involving immune and neural cells being a hallmark feature the loss of myelin sheath enwrapping neuronal axons. Despite recent advances leading to stop the immune system's attack towards oligodendrocytes, remyelinating therapies are still lacking. Remyelination occurs spontaneously in MS patients but it is inefficient and diminishes with disease progression, leading to irreversible disability. Our research aims to better understand the mechanisms leading to efficient remyelination by achieving a deeper understanding of molecular mechanisms leading to

oligodendrocyte generation and differentiation. Genome wide transcriptome comparison of *Olig2 KO* and *Ascl1 KO* together with chromatin immunoprecipitation followed by deep sequencing, led us to uncover Ascl1-Olig2 common direct targets in oligodendrocyte precursors. Among the identified targets, we identified Chd7 (for Chromodomain-Helicase-DNA-Binding 7), a chromatin remodeling protein. We will discuss its expression during oligodendrogenesis both during normal mouse myelination and in remyelination in mouse models and MS tissue. Moreover, we will show its requirement for normal oligodendrogenesis in myelination and remyelination.

S17-03

Daam2-PIP5K is a novel regulatory pathway for Wnt signaling and therapeutic target for remyelination

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Wnt signaling plays an essential role in developmental and regenerative myelination of the CNS, however contributions of proximal regulators of the Wnt receptor complex to these processes remain undefined. To identify components of the Wnt pathway that regulate these processes, we applied a multifaceted discovery platform and found that Daam2-PIP5K comprise a novel pathway regulating Wnt signaling and myelination. Using dorsal patterning of the chick spinal cord we found that Daam2 promotes Wnt signaling and receptor complex formation through PIP5K-PIP_2. Analysis of Daam2 function in oligodendrocytes (OLs) revealed that it suppresses OL differentiation during development, after white matter injury (WMI), and is expressed in human white matter lesions. These findings suggest a pharmacological strategy to inhibit Daam2-PIP5K function, application of which stimulates remyelination after WMI. Put together, our studies integrate information from multiple systems to identify a novel regulatory pathway for Wnt signaling and potential therapeutic target for WMI.

S17-04

The transcription regulator Tcf7l2/Tcf4 in oligodendrocyte development and remyelination

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Constitutive activation of Wnt/β-catenin signaling inhibits oligodendrocyte myelination. Tcf7l2/Tcf4, a DNA-binding transcriptional partner of β-catenin, paradoxically, is required for oligodendrocyte differentiation. How Tcf7l2 modifies β-catenin signaling over development to facilitate myelination processes remains elusive. Here we resolve the paradox by uncovering stage-specific functions of Tcf7l2 through interacting with distinct co-regulators during oligodendrocyte lineage progression. Integrative analysis of genomic occupancy and gene expression profiles reveals that Tcf7l2 interacts sequentially with a competitive inhibitor of β-catenin binding and a differentiation-promoting factor to initiate and sustain oligodendrocyte differentiation, respectively. We further reveal that Tcf7l2 cooperates with differentiation activators to activate the genes encoding enzymes for cholesterol biosynthesis by directly targeting their enhancers. Mice lacking the Tcf7l2 DNA-binding domain exhibit defects in myelination and remyelination after demyelination. Together, our studies identify stage-dependent Tcf7l2-regulated transcriptional switches that promote oligodendrocyte lineage differentiation, indicating a crucial role of Tcf7l2 for homeostatic control of CNS myelination.

SYMPOSIUM 18
Astrocyte metabolic reprogramming by oxygen and energy-dependent pathways: impact on neuronal survival

S18-01

Astrocytic NMDA receptors sustains antioxidant protection of neurons through a novel Cdk5-Nrf2 pathway

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Neurotransmission unavoidably increases mitochondrial reactive oxygen species (ROS) that, when in excess, may be damaging to neurons. Here we found that the antioxidant defense of neurons is weak due to continuous repression, by protein destabilization, of the master antioxidant transcriptional activator, nuclear factor-erythroid 2-related factor-2 (Nrf2). By contrast, Nrf2 is highly stable in astrocytes, explaining their robust antioxidant defense and resistance against oxidative stress. Interestingly, we found that subtle and persistent stimulation of N-methyl-D-aspartate receptors (NMDAR) in astrocytes up-regulates a signal transduction pathway that involves protein kinase C(delta)-mediated stabilization of p35, a cyclin-dependent kinase-5 (Cdk5) cofactor. Active p35/Cdk5 complex in the cytosol phosphorylated Nrf2 at Thr-395, Ser-433 and Thr-439, which was sufficient to promote Nrf2 translocation to the nucleus and induce the expression of antioxidant genes in astrocytes. Furthermore, this Cdk5-Nrf2 transduction pathway was able to induce glutathione metabolism in astrocytes, and to efficiently protect closely spaced neurons against ROS-mediated apoptosis. Together, these results reveal a novel intercellular communication system, through NMDAR, that couples neurotransmission with astrocyte-supported neuronal survival.

S18-02

Regulation of MCT4 expression in astrocytes by both oxygen tension and nitric oxide through a HIF-1α-dependent mechanism

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Monocarboxylate transporters form a small group of H^+-dependent carriers able to transport energy substrates across the plasma membrane of brain cells. MCT4 is a low affinity, high capacity transporter particularly important for lactate release by highly glycolytic cells. In the central nervous system, it is exclusively expressed by astrocytes under physiological conditions. Investigations performed on cultured astrocytes revealed that MCT4 expression is subject to regulation. First, it was observed that reduced oxygen tension leads to a striking enhancement in the expression of MCT4 but not of MCT1. This effect involves a transcriptional regulation as both MCT4 mRNA and protein levels were upregulated by low oxygen exposure. The transcription factor HIF-1α was shown to be necessary and sufficient in this process as the enhancement of MCT4 expression could be prevented by knocking down HIF-1α while it could be mimicked by using DMOG, an inhibitor of prolyl-4-hydroxylase, which leads to HIF-1α stabilization. The implication of AMPK could be excluded as the AMPK activator AICAR was without effect. Enhancement of MCT4 expression was shown to be critical for enhanced lactate release by astrocytes and it was also protective from hypoxic cell death. In addition to oxygen levels, MCT4 expression by astrocytes was also shown to be influenced by a neuroactive signal. Thus, it was demonstrated that nitric oxide (NO) causes a concentration-dependent increase of MCT4 expression in cultured astrocytes. Such an effect was transcriptionally regulated as both MCT4 mRNA and protein levels were enhanced by NO. NO action does not involve the cGMP-Guanylate cyclase pathway, but rather requires the Akt/mTOR/S6 pathway and COX-2-dependent prostaglandin formation. Again, the transcription factor HIF-1α was found to be involved in the induction of MCT4 expression by NO. Interestingly, in addition to MCT4, several genes coding for glycolytic enzymes were also activated in parallel, contributing to the enhanced lactate release observed following NO treatment. Finally, it was shown in co-culture experiments that endothelial cells

represent one putative endogenous source of NO that can upregulate MCT4 and glycolysis in astrocytes. These data demonstrate that the expression of MCT4 in astrocytes (and the associated lactate release) could be regulated in a tonic and phasic manner by distinct signals, suggesting an important role in the control of energy substrate supply to neurons under various situations.

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S18-03

Importance of AMPK for the regulation of astrocyte energy and amino acid homeostasis

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Glucose is the major energy substrate for astrocytes, however, in the brain, astrocytes surround synapses and take up and metabolize glutamate in addition to glucose. Mammalian AMP-activated protein kinase (AMPK) functions as a metabolic switch. In the presence of glutamate, we have shown that activation of AMPK has no effect on glucose uptake in astrocytes, however in the absence of glutamate, it is augmented. In astrocytes, glutamate is either converted into glutamine for release or entering the mitochondria for TCA cycle metabolism. Activation of AMPK did not lead to changes in intracellular glutamate and intra- and extracellular 13C glutamine labeling from 13C glutamate, however in the absence of glucose the conversion from glutamate to glutamine was slightly reduced. This suggests that in the presence of glucose, the essential parts of the glutamate-glutamine cycle, i.e. glutamate uptake, glutamine synthesis and release are not affected by AMPK activation, although glutamate transport and synthesis of glutamine are energy requiring processes. Cataplerosis of TCA cycle intermediates requires the concerted action of malic enzyme (ME) and pyruvate dehydrogenase. We found reduction in the labeling of lactate from 13C glutamate suggesting that AMPK activation may lower the flux of glutamate derived carbon through the ME and lactate dehydrogenase catalyzed reactions. Lactate formation from glutamate is a partial oxidation and degradation of the carbon skeleton and since lactate is released it is an eventual loss of metabolite, thus a catabolic pathway that only partially supports energy production. A reduction in flux to lactate may support an anaplerotic function of glutamate that increases the capacity for glucose oxidation during AMPK activation. Data obtained from incubation of astrocytes with 13C glucose shows that AMPK up-regulates anaplerosis. Such scenario emphasizes that AMPK activation is important to sustain the TCA cycle intermediate pool and in that way increases the capacity for oxidative metabolism of glucose in astrocytes.

S18-04

Astrocyte-Neuron (mis)interactions in Huntington's disease

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The brain makes up 2% of a person's weight. Despite this, even at rest, the brain consumes 25% of the body's energy. Most of the energy consumed in the brain is attributable to restoration of the membrane gradient following neuronal depolarization. Neurotransmitter recycling, intracellular signaling and dendritic and axonal transport also require energy. Even though neurons are responsible for massive energy consumption, the brain is made up of many cells, including neurons, glial and ependymal cells. Every brain cell has a specific function and thus every brain cell has different metabolic needs. Many of these specific functions are concerned with maintainance of neuronal transmission. Astrocytes play a central role in supporting neurons metabolically by producing lactate, through glycolysis and activation of glycogen catabolism. There have been several reports of metabolic impairment in a variety of neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis and Parkinson's disease, among others. Moreover, deregulation of energy metabolism could be implicated in an increased production of oxidative species. During the last 10 years we have been making steady progress in the mechanisms of communication between neurons and glial cells, the way they regulate their metabolism, and the use of ascorbic acid as inter cellular messenger. Here, we will described the regulation of neuronal glucose, lactate and ascorbic acid transporters under synaptic activity in mice models of Huntington's

disease. Experiments demonstrating a failure in astrocytic ascorbic acid recycling and ascorbic acid-dependent modulation on neuronal metabolism in Huntington's disease will be discuss. Brain is an expensive organ in energetic terms so disruptions in energy production may affect neuronal transmission and thus, neuronal survival. FONDECYT1151206,DID-UACh.

SYMPOSIUM 19
Tanycytes - a heterogeneous ependymo-glial cell population with diverse functions in the brain

S19-01

IGF-1 stimulates cell proliferation in a specific area of the hypothalamic wall

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Neurogenesis in the adult rat hippocampal subgranular zone and the subventricular zone of the lateral ventricles has been well documented. Increasing evidence suggests that neurogenesis also occurs in the adult hypothalamus. We report that, after intracerebroventricular treatment with insulin-like growth factor I (IGF-I), cell proliferation significantly increased in both the periventricular and the parenchymal zones of the whole hypothalamus. Proliferation was particularly abundant in a distinct periventricular zone of the caudal hypothalamus. This is an overlapping zone of transition between the cubic ciliated ependyma, dorsally located, and the tanycytic ependyma, ventrally located, where three cell layers can be found: ciliated ependyma, subependyma and tanycytes. After IGF-I treatment, proliferating cells were seen in the subependyma and in the layer of tanycytes. Neurons, astrocytes, tanycytes, microglia and endothelial cells of the local vessels were stained with the proliferative marker 5-bromo-2'-deoxyuridine (BrdU) in response to IGF-I. However, we never observed BrdU-positive ciliated cubic ependymal cells. In the subependyma, proliferating glial fibrillary acidic protein-positive astrocytes contacted the ventricle by an apical process bearing a single cilium and there were many labyrinthine extensions of the periventricular basement membranes. Both features are typical of neurogenic niches in other brain zones, suggesting that the central overlapping zone of the rat hypothalamic wall could be considered a neurogenic niche in response to IGF-I. These effects will be compared with those obtained from studies using other factors.

S19-02

Identity and genetic regulation of neurogenic tanycytes in the hypothalamus

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Hypothalamic tanycytes are radial glial-like cells that emerge during late embryonic/ early postnatal life in rodents. Direct lineage-tracing studies have verified their ability to generate new appetite-regulating neurons postnatally, highlighting hypothalamus as a neurogenic area in the postnatal and adult brain. I will present data that tackles the ensuing questions ie. Which tanycyte subtype acts as the stem cell population? What are the intermediate steps of hypothalamic neurogenesis and what gene/s regulate it. We show that hypothalamic neurogenesis is compartmentalised and beta-tanycytes give rise to new neurons via an intermediate/transient cell population, which resides in the alpha tanycyte domain and has a higher proliferative activity. Moreover, loss of Fgf10 differentially affects the proliferation and trans-compartmental migration of tanycyte subtypes, with a knock-on effect on the number and fate of their daughters, found in the neighbouring parenchymal nuclei. Our studies therefore shed light on the

intermediate steps of hypothalamic neurogenesis, and the differential role of tanycytes subtypes in this process.

S19-03

Tanycytes: the hypothalamic hyperdrive for hormones

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The survival of an organism relies on its ability to promptly, effectively and reproducibly communicate with brain networks that control food intake and energy homeostasis. To achieve this, circulating factors of hunger and satiety reflecting nutrient availability must cross the blood-brain barrier (BBB) to reach effectors neurons. A defect in this process invariably leads to uncontrolled body weight. Here we will discuss the key role played in this process by a peculiar type of glial cells named tanycytes, which have their cell bodies lining the floor of the third ventricle and their endfeet contacting the pial surface of the brain. Recent studies indeed suggest that tanycytes, besides regulating hypothalamic BBB plasticity according to nutrient status, capture metabolic signals such as leptin from the bloodstream and transport them towards their cell body for release into the cerebrospinal fluid. Blockade of this conduit for peripheral metabolic factors into the brain of obese individuals is thought to contribute to the pathophysiology of central hormonal resistance.

S19-04

Tanycyte nutrient sensing and communication in the hypothalamus

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Hypothalamic tanycytes contact the cerebrospinal fluid of the third ventricle, and send processes into the hypothalamic nuclei that control food intake and body weight. Although we have shown, by means of Ca^{2+} imaging, that tanycytes are glucosensitive, the mechanism of sensing remains unresolved. We now have evidence that tanycytes respond to non-nutritive sweeteners known to be ligands of the sweet taste receptor (Tas1r2/Tas1r3 heterodimer). The activation of the sweet taste receptor directly elicits a primary Ca^{2+} signal via a Ca^{2+} influx and release from intracellular stores, which can then lead to a propagating ATP receptor-dependent Ca^{2+} wave.

A receptor for another taste modality - umami - is part of the same gene family as the sweet taste receptor and comprises the Tas1r1/Tas1r3 heterodimer. We now report that L-amino acids such as arginine, lysine, alanine and serine also evokes ATP receptor-mediated Ca^{2+} waves in tanycytes. The agonist potency of the amino acids, and the ability of IMP to enhance these responses, strongly suggests that they are mediated via the Tas1r1/Tas1r3 receptor.

Although both sweet- and umami-signalling by tanycytes evoke ATP receptor-mediated Ca^{2+} waves, the two pathways are mechanistically different. Sweet taste receptors evoke ATP release via pannexin-1 and the resulting Ca^{2+} wave depends only on P2Y1 receptors. The umami receptors evoke ATP release primarily via connexin43. The resulting Ca^{2+} wave requires more than one ATP receptor - the P2Y1 receptor plus the P2X7 receptor and possibly a further P2X receptor.

The physiological importance of nutrient-sensing by tanycytes is unclear. We therefore expressed a Ca^{2+} permeable version of channelrhodopsin2 selectively in tanyctes via an adenoviral construct. We used optogenetic activation to stimulate tanycytes, while recording from arcuate nucleus neurons with patch clamp methods. We find that activation of tanycytes is sufficient to excite neurons, demonstrating for the first time tanycyte-neuron communication. These data strongly suggests that tanycyte sensing of macronutrient status can be communicated to neurons and may thus influence the operation of the hypothalamic networks that control energy homeostasis.

SYMPOSIUM 20
Early glial cell changes in brain degenerative diseases

S20-01

Early microglial activation beyond the substantia nigra in Parkinson's disease

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It is now well established that brain microglial cells contribute to brain homeostasis, and under normal conditions sense their environment. This activity of these cells is enhanced and more focused when pathogens, inflammatory cells or modified proteins occur within the brain. They then turn into another phenotype with concomitant altered function, e.g. phagocytosis and, expression and production of inflammatory mediators and receptors. This is seen under various neurodegenerative conditions, including Parkinson's disease (PD). However, until now most attention has been focused on microglia in the classically affected brain region, i.e. substantia nigra in PD. It has become clear that various other brain regions are affected and express pathological alpha-synuclein which relates to non-motor symptoms in PD, such as autonomic dysfunction, hyposmia and cognitive disturbances. Some of these non-motor symptoms even antedate the motor deficits. In our human post-mortem studies, we questioned whether microglial activation is present in brain regions beyond the substantia nigra, i.e. hippocampus and olfactory bulb. Immunohistochemical analysis has been performed on brain material of PD patients (Braak 4-6), incidental Lewy body disease patients (iLBD; Braak 1-3) and control subjects (Braak 0). Our data show that besides the substantia nigra, microglial activation (CD68) occurs in hippocampus and olfactory bulb of PD patients which is not associated with neuronal cell loss. Moreover, cell proliferation processes (MCM2) in the hippocampus of PD patients are mostly accounted for by microglial cells. When comparing hippocampus and substantia nigra of PD, iLBD and control subjects, phenotypical changes in microglia occurs already in iLBD patients which coincides with elevated expression of toll-like receptor 2 (TLR2) which is considered to contribute to α-synuclein-induced microglial activation. However, in the substantia nigra TLR2 remains present during PD, whereas in the hippocampus, TLR2 is then reduced till control level immunoreactivity. These studies indicate that microglial cells are involved in an early stage of the pathological processes occurring during PD. Moreover, the differential responsiveness of hippocampal versus nigral microglial cells suggest that they are not a uniform cell population throughout the brain but respond differentially to pathological situations in a manner depending on the local environment, and stage of the disease process.

S20-02

Microglial TNF and IL-1 as early disease-modifiers in Alzheimer's-like disease in mice

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In Alzheimer's disease (AD) signs of microglial activation is evident already in prodromal and early AD. This and other evidence suggest that neuroinflammation contributes to the progression of the early disease development in AD. Microglial cells have the capacity to produce cytokines such as TNF and IL-1, and to phagocytose and clear amyloid beta (Aß), however, the influence of TNF and IL-1, and inflammation in general, on these processes is still poorly understood. We have studied the development of Aß pathology, and basal and lipopolysaccharide (LPS) stimulated microglial cytokine production in the $APP_{swe}/PS1_{DE9}$ mouse model of AD. In these mice, cortical Aß plaque load shows a sigmoidal trajectory with age, as it does in AD. At 12 months of age, when Aß pathology is well-developed, TNF and IL-1ß are produced in significantly higher proportions of microglia in the $APP_{swe}/PS1_{DE9}$ mice, than in wildtype mice. Microglial expression of TNF and IL-1ß can be significantly increased by i.p. injection of LPS, which we find reduces cortical Aß pathology at 12 months. Results will also be reported on the influence of IL-1 in modulating Aß pathology during early disease stages in $APP_{swe}/PS1_{DE9}$ mice. Together, the presented work underscores the potential of microglia in modifying

Aß pathology in early AD. Improving the clearance of Aß in prodromal and early AD, might delay or impede the development of AD.

S20-03

The role of astrocyte alterations in early changes in the dynamics of cultured cerebellar networks

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An aberrant response to DNA lesions is implicated in many human brain degenerative disorders. Various types of DNA lesions activate a cellular process known as the DNA damage response (DDR). Mutations affecting the proteins involved in the DDR can lead to severe genomic instability syndromes that involve varying degrees of sensitivity to genotoxic stress, and also to tissue degeneration, cancer predisposition, and premature aging. One of the key components of the DDR is the protein ATM, which is inactivated in the genomic instability disorder ataxia-telangiectasia (A-T). In order to study the effect of malfunctioning DDR on neuronal circuits, we used calcium imaging and immunocystochemical staining to compare the morphology and the dynamics of primary cerebellar cultures grown from postnatal Atm-deficient and wild-type (WT) mice. Cerebellar networks exhibited spontaneous network events after two weeks *in-vitro*. Compared to WT circuits, Atm-deficient circuits displayed a lower number of global synchronizations and a larger number of sparse synchronizations, i.e. synchronous events involving less than a dozen cells. The WT networks display significantly higher level synchronization compared to Atm-deficient networks. In addition, nodes with a high functional connectivity degree were present in the WT networks while in the Atm-/- networks they were not be observed. To understand A-T on the cellular level we tested the hypothesis that A-T is at least partially a glial disease. Immunocystochemical staining of astrocytes revealed a significantly less complex cell arborization in Atm-deficient versus WT circuits, as measured by the number of branches originating from cell bodies. To further study the interrelations between neurons and astrocytes, we generated chimeric networks in which the neurons and astrocytes were extracted from different animals. We found that functional and viable chimera cultures could be prepared only from P8 cerebellar neurons and astrocytes. Chimera cultures made from combinations of P8 cerebellar neurons and P2 cortical glia or from P8 cerebellar neurons and P2 cerebellar glia did not survive and the neurons died within 3 to 4 days of plating. Our results clearly demonstrate that Atm-/- astroglial cell replacement with WT astrocytes fully restores the dynamics of neural networks in chimera neuron-glia networks extracted from Atm-deficient mice. These results support the notion that neuronal network failures in genetic brain degenerative diseases are limited to correlated impairment of astroglial cell functionality.

S20-04

Pathological changes in astrocyte TGFbeta1 levels affect astrocyte-endothelial interactions and trigger cerebrovascular amyloid deposition in Alzheimer's disease

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Astrocyte-endothelial cells (EC) interaction plays a major role in the function of the neurovascular unit. Dysfunction in their interaction may lead to amyloid accumulation on blood vessels, condition known as cerebral amyloid angiopathy (CAA), which can lead to major hemorrhagic stroke, and cognitive impairment. Transforming growth factor beta 1 (TGF-β1) is a pleiotropic cytokine involved both in neurodegenerative and in neuroprotective mechanisms TGF-β1 expression levels correlate positively with the degree of cerebrovascular amyloid in Alzheimer's disease (CAA) cases. Furthermore, expression of TGF-β1 under astrocyte promoter in mice leads to an age-related deposition of amyloid, such as β-amyloid (Aβ), around cerebral blood vessels. We hypothesis that changes in TGF- β1 signaling in endothelial cells, and may affect their role as a part of the blood brain barrier and trigger

CAA pathology. We demonstrated that TGF-β1 affects EC and inflammation cross talk, leading to reduction of secreted factors such as chemokines, which are important for the ability of macrophages to mediate clearance of cerebrovascular amyloid deposition. We showed that activation of macrophages leading to reduction in vascular amyloid, protection from brain damage and improvement in cognition in TGF-β1 mice. We investigated the role of TGF-β1 in facilitating the expression of genes associated with CAA. Using specific endothelial cells gene array we have identified genes that are regulated by specific TGF-β1-smad signaling pathway and play a role in disease progression in mouse model. Understanding the role of astrocyte TGF-β1 in mediating cellular pathways in EC may provide useful therapeutic intervention targets for early diagnosis and treatment of cerebrovascular amyloidosis pathology such as in AD.

SYMPOSIUM 21
The astrocyte-microglial cell nexus

S21-01

The functional impact of astrocyte-microglial cell activation on synaptic function and amyloid pathology

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The primary role of both astrocytes and microglia is to maintain homeostasis and proffer protection to the relatively more vulnerable neurons. There is a great deal of evidence indicating activation of both cells in the brains of aged animals and in the transgenic mouse model of Alzheimer's disease that overexpresses human amyloid precursor protein (APP) and presenilin 1 (PS1; APP/PS1 mice). Thus GFAP expression, as well as several markers of microglial activation, were increased in an age- and genotype-related manner in APP/PS1 mice. In terms of microglia, age- and genotype-related upregulation of markers of the M1, classical activation, state have been described. A key trigger for inducing cells to adopt this state is interferon gamma (IFN gamma) that is derived primarily from peripheral immune cells, including Th1 cells that infiltrate the brain of aged mice and APP/PS1 mice. Interleukin (IL)-4 induces the M2, alternative activation state in microglia but no robust genotype-related changes in markers of M2 activation were identified. Recently, we have found that astrocytes also respond differentially to the polarizing stimuli, IFN gamma and IL-4, albeit to a less profound degree than microglia. Significantly our data show that expression of the IFN gamma receptor is increased on astrocytes prepared from APP/PS1 mice and that the impact of IFN gamma-producing Th1 cells on astrocytes is marked, in terms of production of inflammatory cytokines. We propose that this, together with the reduced glutamine synthetase that occurs with age and following IFN gamma treatment, combine to negatively impact on synaptic function.

S21-02

Astrocyte-derived TGFβ in the regulation of age-related changes in microglia and neurodegeneration

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Question: Aging is the main risk factor for Alzheimer's disease (AD).We hypothesize that AD is caused by an impairment of microglia activation. Astrocytes prevent microglia cytotoxicity by mechanisms mediated by TGFβ1. However, whereas TGFβ1 is increased, cytotoxicity of microglia appears to be especially frequent in aging. Here, we approach this apparent contradiction, assessing if TGFβ1 signaling changes in aging could explain changes on microglial cell function potentiating neurotoxicity.

Methods: We evaluated neurotoxicity of microglia exposed to Aβand the neuroprotective effect of astrocytesand TGFβ1in hippocampal cultures by the TUNEL assay. The participation of the TGFβ1-

Smad3 pathway on the regulation of microglia activation was evaluated at different ages with and without stimulation with LPS and IFNy.The activation of signal transduction pathways, including MAPKs and Smad, as well as modulatory phosphatases like MKP-1, was analyzed.

Results: Astrocytes prevented microglial cell cytotoxicity by mechanisms mediated by TGFβ1, which also affectedAβremoval. Inflammatory activation of astrocytes had a protective effect on Aβneurotoxicity, which depended onthe secretion ofTGFβ. Dissection of the signaling pathways showed thatstimulation with TGFβ1 decreasedIFNy-induced phosphorylation of STAT1 and ERK, which correlated with a reduced production of radical species by glia. Notably, TGFβ1 increased the levels of MAPK phosphatase-1 (MKP-1).TGFβ1 induction and activation of Smad3 signaling by inflammatory stimulation was greatly reduced in adult mice. The reduction on the activation of the TGFβ1 pathway was associated with functional changes on the activation of microglia. Whereas NO secretion predominated on microglia from young mice exposed to LPS, induction of ROS predominated in adult mice. TGFβ1 modulated LPS-dependent induction of NO and ROS production. Modulation was partially dependent on Smad3 pathway and was impaired by inflammatory preconditioning. Phagocytosis was induced by inflammation and TGFβ1 only in microglia cultures from young mice, an effect that was also prevented by Smad3 inhibition.

Conclusions: Astrocytes secrets protective factors regulatingmicrogliacytotoxicity, beingTGFB1 a key regulatory factor.TGFβ1-Smad3 pathway, regulating the activation pattern of microglia, is impaired in agingand by inflammatory preconditioning.Impairment could reduce protective activation while facilitating cytotoxic activation; thus potentiating microglia-mediated neurodegeneration.

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S21-03

Achieving specificity in the astroglial and microglial response to IL-6/gp130 family cytokines

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The IL-6/gp130 family of cytokines includes IL-6, IL-11, leukaemia inhibitory factor (LIF) and oncostatin M (OSM). These cytokines have significant roles in the central nervous system (CNS) including regulating the function of astroglia and microglia in a broad range of processes that span neurodevelopment to neuroinflammation. Although astroglia and microglia are major producers as well as targets of this family of cytokines little is known about the relative roles that these glial cells have in mediating the actions of the different IL-6/gp130 cytokines and if and how there is biological specificity in the response of these cells to these cytokines. Microarray studies by us revealed that in addition to overlapping responses, these glial cells can exhibit unique responses to the same cytokine despite sharing common signal transduction pathways. The binding of the IL-6/gp130 cytokines to their cognate receptors triggers signal pathway activation via gp130 and phosphorylation of STAT1 and STAT3 as well as the ERK/MAPK pathway. We have found that multiple molecular mechanisms underlie the responses of astroglia and microglia to IL-6/gp130 cytokines that contribute to biological specificity. These include, differential expression of specific receptors (e.g. microglia have IL-6Ra but astroglia do not while the opposite occurs for OSMRb) and varying levels of activation of STAT1, STAT3 and ERK depending on the cytokine involved. The neurobiology of IL-6 is complicated further by the fact that while astroglia lack IL-6Ra these cells still respond to the combination of IL-6 plus the soluble IL-6R in a process know as trans-signaling. Using a transgenic mouse model with the production of IL-6 targeted to astrocytes we found that trans-signaling is responsible for many of the detrimental actions of this cytokine in the CNS.

S21-04

Astrocyte targeted production of either IL-6 and IL-10 promotes alterations in microglial activation and has a direct effect on neuronal survival after peripheral nerve axotomy

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When the integrity of the nervous tissue is disturbed, glial cells become quickly activated. Accumulating evidence indicate that among the different inflammatory mediators involved in glial activation, cytokines are the molecules that exert a more rapid and direct action on them modulating their response. In this regard, the regulatory cytokine Interleukin 6 (IL6) and the counterregulatory cytokine Interleukin 10 (IL10) play key roles in maintaining the required pro- and anti-inflammatory balance, which is critical to prevent both glial reactivity and the inflammatory process getting out of control. In order to better clarify the role of these cytokines in microglial and astroglial activation, we investigated the local effects exerted by astrocyte-targeted production of either IL6 or IL10 after peripheral nerve transection. To accomplish this, we have used either GFAP-IL6 or GFAP-IL10 transgenic mice (IL6 tg and IL10 tg, respectively) and the facial nerve axotomy paradigm. Our observations demonstrated that local upregulation of these cytokines, in concomitance with astrocyte reactivity, promotes changes in microglial activation impacting the survival of axotomyzed motor neurons in the facial nucleus (FN). In comparison with their respective wild types, in IL6 tg, in parallel with increased neuronal death, activated microglia displayed, among others, increased MHC-II and CD16/32 but decreased CD86, CD18, CD11b and CD49e. In contrast, in IL10 tg, in parallel with reduced neuronal death, microglia showed an high increase (even more than IL6 tg) not only in MHC-II and CD16/32 but also in CD86 and CD18. Noteworthy, the largest number of FN surviving motor neurons in IL10 tg coincides with increased osteopontin and CD44 expression and a high number of microglial clusters. In conclusion, our studies showed that astroglial targeted production of IL6 and IL10 has a direct impact on the microglial response and motor neuron survival after facial nerve axotomy. Ongoing studies to investigate the alterations produced by these cytokines in the specific crosstalk between microglia, astroglia, injured neurons and infiltrating lymphocytes will provide us with a better understanding of molecular signalling mechanisms involved in regulation of glial reaction and neuronal death.

SYMPOSIUM 22
Molecular and cellular changes in CNS myelinating glia during demyelination and remyelination

S22-01

Deconstructing and reconstructing the perinodal area in demyelination and remyelination

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The segregation of myelinated fibers into distinct domains around the node of Ranvier - the perinodal areas - is crucial for efficient nerve conduction. Perinodal areas are formed by interactions of molecules between the axon and the myelinating glia. In a variety of demyelinating pathologies including multiple sclerosis, the molecular architecture of the myelinated fiber is disrupted, leading to axonal degeneration. A detailed analysis of the alterations of Contactin-2/TAG-1, Caspr2 and voltage-gated potassium channels (VGKCs), forming the juxtaparanodal tripartite complex, in relation to adjacent paranodal and nodal molecules, will be presented in two different models of CNS demyelination, the experimental autoimmune encephalomyelitis (EAE) and the cuprizone model of toxic demyelination. Extensive alterations of the juxtaparanodal molecular architecture have been found under de- and remyelinating conditions. Inflammation alone was sufficient to disrupt the borders between the domains leading to the diffusion of juxtaparanodal components to the adjacent paranodal area. EAE induction and cuprizone-induced demyelination resulted initially in paranodal domain elongation with subsequent diffusion of the juxtaparanodal components and the reduction of their expression levels. At later stages, with decreasing inflammation and spontaneous remyelination there was a partial restoration of the paranodal domain but not sufficient re-organization of the juxtaparanodes. The latter were re-formed only when complete remyelination was allowed in the cuprizone model, indicating that juxtaparanodal domain re-organization is a later event that may remain incomplete in a hostile inflammatory milieu. Our results showing juxtaparanodal alterations in

these two models add new information to the working model of perinodal domain distribution under demyelinating conditions (summarized in the scheme below).

S22-02

Promoting recovery after premature brain injury: a role for enhanced epidermal growth factor receptor signaling

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There are no effective treatments available that improve function in the growing population of very preterm infants (<32 weeks gestation) with neonatal brain injury. Diffuse white matter injury (DWMI) and gray matter involvement are common long-term findings in these children and contributes to their chronic neurodevelopmental and cognitive impairment. Using sublethal chronic perinatal hypoxia (Hyp; postnatal day P3-11) as a clinically relevant mouse model of very preterm brain injury, we have recently demonstrated that administration of intranasal epidermal growth factor (HB-EGF; P11-14) stimulates a response of endogenous EGF receptor (EGFR)-expressing progenitor cells in the white matter and promotes cellular, biochemical and functional recovery. It is established that injury results in a significant expansion of the neural stem cells (NSCs) and progenitor cells that reside in the subventricular (SVZ) and the hippocampal dentate gyrus subgranular zone (SGZ). However, it is unknown whether administration of intranasal HB-EGF treatment after Hyp: i) provides an additive stimulatory effect on newly generated NSCs and progenitor cells in these regions; and ii) results in a differential response in the SVZ and SGZ. In this study, we demonstrate that, in both the SVZ and SGZ, Hyp induces a significant increase in: i) phosphorylated-EGFR and EGFR-ligand expression immediately after Hyp, but not at later time points; ii) the number of proliferating cells; iii) the number of Pax6-expressing (neurogenic) and Ascl1-expressing (bipotential) progenitor cells; and iv) the number of NG2-expressing oligodendrocyte progenitor cells (OPCs). In the SVZ, HB-EGF treatment after Hyp resulted in a significant expansion of Sox2-expressing progenitor cells, and prevented Hyp-induced expansion of Pax6-expressing cells and doublecortin (DCx)-BrdU-positive cells. Conversely, in the SGZ, HB-EGF treatment had an additive effect on both DCx-BrdU-positive cells and newly generated neuronal NeuN-positive cells. Further studies using fate-mapping techniques with tamoxifen-inducible Cre-transgenic mice are being performed. Our results indicate that HB-EGF treatment has differential effects in the SVZ and SGZ, in particular on neuroblast formation. Understanding the benefits of this differential effect on functional outcome after developmental brain injury is crucial in order to develop intranasal HB-EGF treatment as a potential therapeutic strategy for neonatal brain injury caused by very premature birth.

S22-03

Regulation of synaptic connectivity of oligodendrocyte precursor cells in myelination and myelin repair

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Oligodendrocyte precursor cells (OPCs), also named NG2 cells, constitute the major source of myelinating oligodendrocytes during development and in demyelinating diseases. Moreover, OPCs receive functional and morphological synaptic inputs from neurons in grey and white matter. However, the operating modes and functions of these synapses during myelination and myelin repair are unknown.

In this talk, I will describe our new findings on the regulation of OPC synaptic connectivity during myelination and myelin repair. In a first study, we analysed the synaptic connectivity between GABAergic interneurons and OPCs of the developing somatosensory cortex, during the most critical days of neocortical OPC differentiation. We brought into play new approaches in the field of OPC synaptic physiology to explore unitary neuron-OPC synapses: paired recordings and circuit mapping with glutamate (holographic) photolysis. We unexpectedly found that the GABAergic innervation of OPCs by cortical interneurons is temporally and spatially regulated from the subcellular to the network

level in coordination with the onset of oligodendrogenesis. In a second study, we assessed the properties of OPC glutamatergic synapses in demyelinating conditions using a model of lysolecithin (LPC)-induced focal demyelination of *corpus callosum*. By combining electrophysiology and immunostainings in different transgenic mice expressing fluorescent reporters, we showed that newly generated OPCs inside the lesion are born without synapses during their active phase of proliferation, but gain synaptic inputs during remyelination.

In conclusion, OPC synaptic connectivity is highly regulated in time and space during brain development and in pathological conditions. This regulation is correlated with important OPC developmental processes, suggesting the implication of these neuron-glia synapses in the fate of OPCs.

S22-04

Junctions and gaps: glial connexins in Multiple Sclerosis and EAE

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Glial cells are coupled extensively through gap junctions (GJs) formed by cell type-specific connexins. Inherited human disorders affecting glial connexins and respective animal models have highlighted the crucial role of GJ connectivity for CNS myelination and homeostasis suggesting that they may also be involved in acquired CNS demyelination and remyelination. We have studied GJ alterations in post-mortem brain samples from multiple sclerosis (MS) patients as well as during the course of experimental autoimmune encephalomyelitis (EAE) in wild type and GJ deficient mice. Using morphological, biochemical, and gene expression studies we found significant alteration in glial connexins not only in MS lesions, but also extending into the normal appearing white matter (NAWM). The oligodendrocyte connexins Cx32 and Cx47 were reduced in and around chronic MS lesions, while Cx43, the major astrocytic partner in oligodendrocyte-astrocyte (O/A) GJs, was increased, reflecting astrogliosis. Cx47 was expressed in oligodendrocyte precursor cells (OPCs), but OPCs showed only limited connectivity to reactive astrocytes. These alterations were replicated in the EAE model, with persistent loss of Cx47 and Cx32 GJs even away from lesions. During acute EAE Cx43 was severely reduced with disruption of O/A GJs. In chronic EAE, as in chronic MS brain, Cx43 expression was increased with astrogliosis, but without complete reestablishment of GJs to oligodendrocytes. EAE clinical severity and CNS pathology are exacerbated in Cx32 or in Cx47 knockout mice, indicating increased vulnerability of myelin and axons. Further studies in MS cortex showed Cx32 and Cx47 GJ loss within cortical lesions and in normal appearing grey matter (NAGM). In contrast, cortical astrocyte connexins Cx30 and Cx43 showed increased expression accompanied by diffuse microglial activation and marked astrogliosis of the cortex. Increased Cx43 expression correlated with inflammatory load while Cx32 expression correlated with longer disease duration and therefore milder course. Thus, disrupted oligodendrocyte GJ connectivity and disconnection from reactive astrocytes in white and gray matter in the setting of persistent inflammation and astrogliosis emerge as important aspects of both focal and diffuse MS pathology and may play a role in failed remyelination and disease progression.

SYMPOSIUM 23
Astroglial connexins in brain functions and pathologies involving neuro-glio-vascular interactions

S23-01

Astroglial hemichannels contribute to neuronal suffering in a mouse model of Alzheimer's disease

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Astrocytes, that closely interact with neurons, contribute to neuronal viability and their dysfunction is associated to many brain pathologies such as neurodegenerative diseases.This is the case for Alzheimer's disease (AD) in whichb-amyloid peptides (Ab) play a critical role, modify glial phenotypes and exert toxic effects on neurons. Moreover, astrocytes represent the brain cell population that expresses the larger number of connexins, the molecular constituents of gap junction channels and hemichannels.In two murine models of Alzheimer's disease (APP/PS1 mice) the expression of Cx43 and Cx30, the two main astroglial Cxs, is increased in protein extracts from brain hemispheres with an onset at 9 months. In a large majority of amyloid plaques, an enhanced immunoreactivity is also detected for both Cxs. This elevated Cx immunoreactivity is concentrated in astroglial processes infiltrating the plaques and is not detected in activated microglia. Similar observations are obtained in brain samples from AD patients. Functional tests indicate that hemichannel activity is increased in astrocytes from APP/S1 mice. This feature is maintained in APP/PS1 mice crossbred with Cx30 KO mice while it is inhibited when crossbred with astroglial targeted Cx43 KO mice. A pharmacological analysis confirms a major contribution of Cx43 to hemichannel activity. Moreover, while in the hippocampus of APP/PS1 mice (8-9 months) theaccumulation of mitochondrial superoxide ions (Mitosox) in neurons and the presence of dystrophic neurites (revealed by RTN3 immunostaining), indicateneuronal suffering,these two parameters are greatly reduced in the APP/PS1 crossbred with the Cx43 KO mice. Finally, chronic (3 months) treatment of APP/PS1 mice with a hemichannel inhibitor delivered daily in the drinking water also results in a significant reduction of neuronal damage markers (Mitosox and RTN3).Altogether, these findings open the way for future alternative therapeutic strategies that identify astroglial hemichannels constituted by Cx43 as a potential target to fight against neurodegenerative processes in AD.

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S23-02

Regulation of glia cell hemichannels during stress

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Introduction: Gestational stress activates microglial cells in the brain of fetuses, condition that persist in the offspring. However, inflammasome activation in oligodendrocytes induced by prenatal stress in the offspring remains unknown. Moreover, microglial cells act as sensor of adverse conditions including stress and viruses. The latter are the most common cause of infections during prenatal life (detected via TLR3 that recognizes viral double-stranded RNA or the mimetic, poly (I:C)). we hypothesize that "stress potentiates the increase in glial cell hemichannel activity induced by poly (I:C)".

Methods and Results: Offspring (1 day old) of control and dexamethasone treated (last 5 day of gestation) mice were used. In oligodendrocytes of stressed mice, levels TLR4, CD14, $P2X_7R$, TNF-α, Panx1, Cx29, Cx32 mRNAs were increased while level or Cx47 mRNA were reduced and levels of IL-1β and iNOS were not affected. Nonetheless, conditioned medium of astrocytes or microglia from stress animals increased the levels of all molecules mentioned above with the exception of Cx47 that was reduced. In brain slices, stress enhanced the ethidium uptake (snap shots and labeling of specific

cell markers detected by confocal microscopy) in oligodendrocytes and was inhibited by Panx1 and Cx43 hemichannel blockers as well as by inhibitors of $P2X_7R$ and CRHR1 and mast cells. These responses were not reversed in pups maintained for 60 days with none stressed mothers.

Primary microglia cultured stimulated with poly I:C (10 mg/ml) for 48 h presented elevated levels of CD86 and MHC II, increased alostimulatory capacity on T lymphocytes and elevated hemichannel activity (etidium uptake, time lapse). Moreover, the ethidium uptake of microglia and mast cells present in brain slices was also evaluated. In stressed animals, the hemichannel activity of microglia was evident under basal conditions and poly (I:C) enhanced the hemichannel response. Moreover, the response to poly (I:C) was earlier in brain slices of stressed than in control mice. Notably, mast cells responded later than microglia both in stressed and control mice.

Conclusions: Stress activates the inflammasome in oligodendrocytes via CRHR1 (Mast cells)-Panx1 (Microglia and Astrocytes), connexin43 (Astrocytes) and this response persists at least for several weeks and thus might be a relevant factor that alters perinatal neurogenesis.

Therefore, hemichannels of microglia could be a novel target with clinical applications in brain alterations derived of stress and viral infection.

S23-03

Connexin and Ca^{2+} signaling in glial and endothelial cells is implicated in inflammation-induced blood-brain barrier permeability changes in vivo

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Situated between the circulation and the brain, the blood-brain barrier (BBB) protects the cerebral tissue from circulating toxins while securing a specialized environment for neuro-glial signaling. BBB function is maintained by signaling interactions with blood cells, astrocytes, pericytes and neurons that occur in the neurogliovascular unit. BBB integrity is compromised in a multitude of pathologic conditions, including exposure to systemic endotoxins (e.g. lipopolysaccharide - LPS) that are associated with an inflammatory response and adverse effects on nervous tissue functioning. We here used intraperitoneal injection of LPS in mice to trigger an in time and severity expanding increase in BBB permeability. Previous work from our lab demonstrated that the endothelial cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) is an important factor determining the functional state of the BBB and that connexin hemichannels (CxHCs), small aqueous pores in the plasma membrane, contribute to $[Ca^{2+}]_i$ dynamics and BBB alterations. We here demonstrate that the LPS-induced BBB permeability increase is prevented by intravenous injection of the Ca^{2+} chelator BAPTA-AM and of Cx channel blocking peptides *in vivo*. At the abluminal side, the BBB endothelium is in contact with astrocytic endfeet and we find that buffering astrocytic $[Ca^{2+}]_i$ changes and blocking astroglial CxHCs by applying BAPTA-AM or Cx channel-targeting peptides directly to the exposed cortex prevents the LPS-induced barrier breach. Work with inducible Cx43 knockout mice and mice carrying an astrocyte-specific deletion of Cx43 confirmed the protective potential of Cx-targeting strategies and pointed to a crucial role of Cx43 as a target. Collectively, our results demonstrate that BBB malfunctioning in response to inflammatory mediators is mediated by $[Ca^{2+}]_i$ dynamics and CxHC signaling in both endothelial cells and astrocytes. Further work is needed to establish the role of the signaling pathways in astrocyte-endothelial interactions, but our results bring up Cx channels as interesting targets with therapeutic potential.

S23-04

Connexin43 and Src interaction in glioma stem cells

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Connexin43 (Cx43) as a building block of gap junction channels and hemichannels exerts important functions in astrocytes. When these cells acquire a malignant phenotype Cx43 protein levels are downregulated, being negligible in high-grade astrocytoma or glioblastoma multiforme, the most common and deadliest of malignant primary brain tumors in adults. These tumors are composed of a heterogeneous population of cells that includes many with stem-cell-like properties, called glioma stem cells (GSCs) or glioma initiating cells, which are highly tumorigenic and lack Cx43 expression. Interestingly, restoring Cx43 reverses GSC phenotype and consequently reduces their tumorigenicity. Our goal was to investigate the mechanism by which Cx43 exerts this antitumorigenic effect on GSCs. We focused on the intracellular carboxy tail of Cx43, which interacts with several signaling proteins, including the tyrosine kinase c-Src. Our results show that restoring Cx43 in GSCs inhibited c-Src activity, which in turn promoted the down-regulation of the inhibitor of differentiation Id1. Id1 sustains stem-cell phenotype since it controls the expression of Sox2, responsible for stem cell self-renewal, and promotes cadherin switching, which has been associated to epithelial-mesenchymal transition. Our data suggest that Cx43, by inhibiting c-Src, down-regulates Id1 with the subsequent changes in stem-cell phenotype, since both the ectopic expression of Cx43 and the inhibition of c-Src reduced Id1, Sox2 expression and promoted the switch from N- to E-cadherin. Based on this mechanism, we have designed a cell-penetrating peptide, containing the region of Cx43 that interacts with c-Src (CPP-266-283) that mimics the antitumorigenic effect of Cx43 on GSCs. These data confirm the relevance of the interaction between Cx43 and c-Src in the regulation of the malignant phenotype and pinpoint this interaction as a promising therapeutic target.

SYMPOSIUM 24
Schwann cell biology of CMT neuropathies

S24-01

Protein quality control pathways in PMP22-linked neuropathies

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Duplications or deletions in the peripheral myelin protein 22 (PMP22) gene account for the majority of Charcot-Marie-Tooth neuropathies. PMP22 is a 22 kDa hydrophobic tetraspan Schwann cell protein with low folding efficiency and a substrate for proteasomal degradation. Imbalance in the amount of PMP22 destined for degradation leads to accumulation of misfolded proteins in the cytosol, a decline in proteasome activity, and ultimately, pathology within the Schwann cell. Accordingly, we formulated the hypothesis that protein quality control mechanisms, namely autophagy and chaperones, are candidates for therapueitc development via their potential toprovide alternative pathways for the processing of PMP22.Pharmacologic modulation of autophagy by rapamycin (RM) was evident *in vitro,* with a reduction in PMP22 aggregate formation and improved myelination in neuron-Schwann cell explant cultures. RM was less effective *in* vivo, failing to alleviate the phenotype in neuropathic Trembler J mice. Surprisingly, RM-induced activation of autophagy within the target tissues was beneficial to peripheral nerves but not to skeletal muscle of affected mice. As an alternative strategy, we screened several small molecule inhibitors of HSP90 to enhance chaperone expression and improve myelin formation by neuropathic Schwann cells. The small molecule AUY922 emerged as a leading candidate for further testing. Two month old wild type and PMP22-overexpressing C22 mice were randomly assigned to cohorts that received either two weekly injections of placebo or AUY922 (2 mg/kg) with body mass and locomotor performance used as outcome measures throughout a 4-month long study. AUY922 significantly benefited rotarod performance of treated mice, suggesting improvement in neuromuscular performance. These results were supported by significant increases absolute and specific peak force produced by the tibialis anterior muscle following nerve stimulation. Morphological analyses of sciatic nerves demonstrated preservation of myelinated nerve fibers and improved processing of PMP22 following AUY922 treatment. Our results support the development of the chaperone pathway as a therapeutic target in CMT1A neuropathies, and recommend the identification of alternative approaches in targeting the autophagic pathway.

S24-02

Endoplasmic reticulum stress and hereditary neuropathies

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Endoplasmic Reticulum (ER) Stress has been implicated in the pathogenesis of both acquired and hereditary neuropathies. For example, mutant proteins associated with several Charcot-Marie-Tooth (CMT) neuropathies provoke ER stress and an unfolded protein response (UPR), and perturbations of UPR alter the severity of neuropathy. P0 glycoprotein is abundantly synthesized in myelinating Schwann cells. The mutant P0S63del causes CMT1B neuropathy in humans, and a very similar demyelinating neuropathy in S63del transgenic mice. P0S63del is retained in the ER of Schwann cells where it promotes unfolded protein stress and elicits an UPR associated with translational attenuation. We have shown that ablation of CHOP, a UPR mediator downstream of the PERK kinase sensor of unfolded proteins, ameliorates demyelination in S63del nerves. In addition, Gadd34 is a detrimental effector of CHOP that reactivates translation too aggressively in myelinating Schwann cells. Limitation of Gadd34 function improves myelination in S63del nerves, and reduces accumulation of P0S63del in the ER by maintaining translational attenuation. Surprisingly, the PERK ablation in Schwann cells, which activates translation and therefore should worsen demyelination, actually improves myelination and neuropathy. PERK may have additional targets outside of the UPR. Limiting Gadd34 in order to reset translational homeostasis may provide a therapeutic strategy in tissues impaired by misfolded proteins.

S24-03

Phospholipid metabolism, the regulation of membrane trafficking and Charcot-Marie-Tooth neuropathies

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Phosphoinositides (PIs) phospholipids are seven different intermediates, which derive from the poly-phosphorylation of the phosphatidylinositol, the precursor molecule. PIs have a restricted distribution at intracellular membranes, which is strictly controlled by the concerted action of opposing kinases and phosphatases. Given the fast turnover and regulated dynamics, PIs represent potent signalling molecules that govern a variety of cellular functions including membrane trafficking.

The most known PI-mediated signalling is the PtdIns(3,4,5)P_3-AKT-mTOR pathway, in which PtdIns(3,4,5)P_3, also known as PIP_3, is generated by activation of PI3K class I and dephosphorylated by the PTEN phosphatase. It is thus not surprising that the PI3K-PtdIns(3,4,5)P_3-AKT-mTOR pathway has been demonstrated as one of the main signalling promoting myelination in Schwann cells. Interestingly, enzymes regulating PI metabolism and other proteins, which rely on PI for their functions, have been recently found mutated in Charcot-Marie-Tooth neuropathies.

We will discuss how mutations in these PI-associated proteins impact on the regulation of endocytosis, recycling, late endosomal trafficking and lysosomal mediated degradation. All together, these studies support the notion that the regulation of intracellular trafficking is a key process in Schwann cell biology.

S24-04

Experimental therapy of Charcot-Marie-Tooth disease 1A with soluble Neuregulin-1

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A duplication of the gene encoding the peripheral myelin protein of 22kDA (PMP22) defines the most common inherited neuropathy, Charcot-Marie-Tooth disease 1A (CMT1A), a disease without known cure. Although demyelination represents a characteristic disease feature, the clinical phenotype of CMT1A is determined by the degree of axonal loss, and patients suffer from progressive muscle weakness and impaired sensation. CMT1A disease manifestation is thought to begin in young adulthood, however walking disabilities, foot deformities and electrophysiological abnormalities are already present in childhood. We show in *Pmp22* transgenic rodent models of CMT1A, that Schwann cells acquire a persistent differentiation defect during early postnatal development, caused by an imbalanced activity of the PI3K/AKT and the MEK/ERK signaling pathways.

The soluble isoform Neuregulin-1 (NRG1) type I, expressed by Schwann cells, has recently been shown to be required for Schwann cell redifferentiation and remyelination after peripheral nerve injury. We will show how enhanced PI3K/AKT signaling by axonally overexpressed Neuregulin-1 (NRG1) type I drives diseased Schwann cells towards differentiation and preserves peripheral nerve axons. In a preclinical experimental therapy using a CMT1A rat model, soluble NRG1 effectively overcomes impaired peripheral nerve development and restores nerve function until adulthood, even when treatment is restricted to early postnatal development. Our findings suggest a model in which Schwann cell differentiation within a limited time window is crucial for the long-term maintenance of axonal support. As a perturbed Schwann cell differentiation phenotype is not specific for CMT1A but has also been reported for other inherited peripheral neuropathies, this strategy may potentially constitute a common therapeutical approach for demyelinating CMT neuropathies.

SYMPOSIUM 25
Glial heterogeneity and reprogrammability

S25-01

In vivo reprogramming of striatal NG2 glia into functional neurons that integrate into local host circuitry

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The possibility to directly convert non-neuronal cells residing in the brain into neurons *in situ* opens up the possibility for new therapies, aiming at repairing the brain after injury or degenerative disease. We here show direct conversion of NG2 glia in the striatum of adult mice into neurons by the forced expression of the three transcription factors Ascl1, Lmx1a and Nurr1 using AAV-vectors. Using a new reporter system that allows for long term monitoring of the reprogrammed neurons, we show that the converted neurons remain stable over a long time, progressively start to exhibit a mature neuronal morphology, down-regulate glia specific properties and express mature neuronal markers. 12 weeks after conversion, the neurons display electrophysiological properties of functional neurons, and monosynaptic tracing based on modified rabies vectors show that the new neurons integrate into local circuitry in an efficient manner.

S25-02

In vivo reprogramming of adult glia to neural progenitors and mature neurons

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Injury to the central nervous system leads to irreversible neuron loss and the formation of a glial scar. This scar is initially beneficial for restricting secondary damage but ultimately inhibitory for neural regeneration. Strategies remodeling the scar-forming glial cells might enhance regeneration and

promote functional recovery after neural damage. We have examined the possibility of converting reactive astrocytes, a major cell type forming the glial scar, to neurons in the adult brain.

Endogenous reactive astrocytes were specifically targeted by lentivirus-mediated gene expression under the human GFAP promoter. Our in vivo screens showed that the transcription factor SOX2 alone can induce the appearance of neural progenitors within the adult or even aged mouse striatum. Fate mapping showed that these induced neural progenitors were generated from astrocytes. Importantly, the induced progenitors can generate mature neurons when additional signaling molecules, such as brain-derived neurotrophic factor (BDNF), BDNF/Noggin, or valproic acid (VPA), were applied. Electrophysiological recordings demonstrate that astrocyte-converted neurons can integrate into the local neuronal network in the adult brain. Astrocytes can be similarly converted into mature neurons in the adult spinal cord after injury.

Together, our results reveal a brand new strategy to regenerate the damaged central nervous system, which is to reprogram the reactive glial cells to functional neurons. This proof-of-concept study may have important implications in regenerative medicine by using a patient's own cells without the need of transplantation.

S25-03

Identification and successful negotiation of a metabolic checkpoint in direct neuronal reprogramming

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Direct reprogramming of somatic cells into different lineages allows generation of specific cell types that cannot otherwise be easily harnessed, which is particularly important for cells from organs not readily accessible, such as neurons from the central nervous system. This raises the exciting possibility of reprogramming-based therapies that may replenish neuronal populations using glial cells surrounding an injured area *in vivo.* Despite the importance of direct neuronal reprogramming, the cellular and molecular mechanisms underpinning fate conversion remain largely unknown. Using continuous single cell live imaging we identified a critical point after which cells either successfully converted into neurons or succumbed to cell death. Investigating factors that improved negotiation of this checkpoint revealed oxidative stress-reduction as an important forskolin-induced mediator of reprogramming enhancement. Genome-wide expression and redox state analysis during reprogramming identified key genes and pathways that mediated metabolic transitioning and reducing lipid peroxidation downstream of forskolin. These findings highlight the importance of the metabolic transition from a typically glial glycolytic-metabotype to a mitochondrial-dependent metabolism characteristic of postmitotic neurons. Such pathways lead to an unprecedented improvement in glial-to-neuron conversion after traumatic brain injury *in vivo*.

S25-04

In vivo reprogramming: reversing glial scar back to neural tissue for brain repair

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Glial scar is a common pathological hallmark that is widely associated with brain injury, stroke, glioma, and neurodegenerative disorders such as Alzheimer's disease. Reactive glials initially exert neuroprotective effects but later form glial scars to inhibit neuronal growth. Currently, there is no effective way to reverse glial scars back to normal neural tissue. We have recently developed an innovative *in vivo* reprogramming technology to directly convert reactive glial cells into functional neurons inside the mouse brain (Guo et al., Cell Stem Cell, 2014). This is achieved through *in vivo* expression of a single neural transcription factor NeuroD1 in the reactive astrocytes in injured mouse brain or model animals for Alzheimer's disease. Our *in vivo* direct cell conversion technology will not

only reduce the number of reactive astrocytes, but also generate new neurons simultaneously at the injury site, making it possible for the first time to reverse glial scar back to neural tissue. Such internal trans-differentiation method will avoid immunorejection and tumorigenesis associated with conventional stem cell therapy. We have further demonstrated that cultured human astrocytes can be directly converted into functional neurons by small molecules, suggesting that our cell conversion technology may be developed into drug therapies for human brain repair.

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SYMPOSIUM 26
Nuclear receptors in CNS inflammation, demyelination and repair

S26-01

Experimental and clinical effects of PPARγ modulating drugs in Alzheimer's disease

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Activators of PPARγ including endogenous ligands as well as sythentic compounds have been suggested to exert beneficial effects in cellular and rodent models of neurodegenerative disorders. Several non-steroidal antiinflammatory drugs, such as ibuprofen, indomethacin and naproxen may reduce the risk to develop Alzheimer disease through activation of PPARγ. Suppression of inflammation, modulation of APP processing and improved microglial phagocytosis may represent key mechanisms involved in the epidemiologically observed protection. Molecular mechanisms will be reviewed and interactions with other nuclear hormone receptors being discucssed. New data from Cre-lox modified neuronal PPARγ ko models will be presented revealing mechanisms of action of non-steroidal antiinflammatory drugs (ibuprofen) and thiazolidinediones (pioglitazone). Additionally, mechanisms responsible for PPARγ dysfunction during the course of Alzheimer's disease will be uncovered. Likewise, new epidemiological data will be presented, revealing a substantial preventive protection from dementia in type II diabetic patients treated with the PPARγ activating drug pioglitazone.

S26-02

Role of LXRα and β in the myelination of the central and peripheral nervous system: cross-talk with Wnt/beta catenin pathway

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Myelination is a complex and fine tuned process governed by several signaling pathways. Our aim is to discover new signaling pathways impacted in myelination and remyelination. First, we have shown that Wnt/beta catenin is crucial for the initiation of expression of myelin gene expression in both the central and peripheral nervous sytems. We have also studied the effect of oxysterols, which are reactive molecules generated from the nervous system. Our aim was to study the influence of oxysterols on myelin gene expression and myelin sheath formation by oligodendrocytes (central nervous system), and by Schwann cells (peripheral nervous system). We show by gas chromatography/mass spectrometry that oligodendrocytes and Schwann cells contain 24(S)-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol, and that they express their biosynthetic enzymes and receptors (Liver X Receptors: LXRα and LXRβ). We demonstrate that oxysterols activate the expression of central myelin genes (PLP and MBP) in oligodendrocytes, but inhibit peripheral myelin genes expression (MPZ, PMP22) in a Schwann cells.

In the CNS, we showed that the activating effects of oxysterols were restricted to the cerebellum and dependant of LXR presence as myelin gene expression was drastically reduced in this structure in LXR a/b KO mice. By, using organotypic cultures of cerebellum slices, we showed that oxysterols

were able to stimulate myelin gene expression after lysolecithin-induced demyelination and enhance remyelination process.

In the PNS, the down-regulation of myelin genes is mediated either by LXRα or LXRβ, depending on the promoter context. Importantly, the knockout of LXR in mice results in thinner myelin sheaths surrounding the axons of the nerves. Oxysterols repress peripheral myelin genes via two mechanisms: by binding of LXRs to myelin gene promoters and by inhibiting the Wnt/beta-catenin pathway that is crucial for the expression of myelin genes.

Altogether our results reveal new mechanisms of action of oxysterols and their dialogue with Wnt/beta catenin pathway, and open new perspectives for the treatment of demyelinating diseases by targeting LXR.

S26-03

Two novel nuclear receptors in glial cell function

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Estrogen receptor beta (ERβ) and Liver X receptor beta (LXRβ) are two ligand-activated nuclear receptors which were unknown before 1996. Both receptors have been found to play distinct and important roles in glial cells and, because they are ligand-activated transcription factors, they are extremely suitable as targets for treatment of diseases involving defective glial cells. Selective ERβ ligands have been developed by pharmaceutical companies and, because they do not activate ERα, they do not increase the risk of breast cancer, do not feedback regulate the pituitary and do not cause unwanted cardiovascular effects which accompany the use of estrogen. So far no LXRβ-specific ligands have been developed.

ERβ is expressed in microglia and oligodendrocytes but not in astrocytes while LXRβ is expressed in astrocytes and microglia. Both receptors are suppressors of microglial activation and their ligands are being investigated as pharmaceuticals in the treatment of neuroinflammation. Of particular interest is the phenotype of LXRβ-/- mice in which larger motorneurons in the spinal cord and dopaminergic neurons in the substantia nigra degenerate spontaneously after mice are 7 months old. By one year of age the male, but not female LXRβ ko mice develop hind limb paralysis. LXRβ is not expressed in motor or dopaminergic neurons and it appears that the etiology of the neuronal loss is defective immunomodulation of microglia.

ERβ-selective ligands are being studied as anti neuroinflammatory agents and as drugs that may induce re-myelination after axonal injury. They have been shown to be effective in mouse models of multiple sclerosis and Parkinson's disease.

Thus these two novel nuclear receptors can be regarded as new players in the arena of neurodegenerative diseases which include Alzheimer's disease, Parkinson's disease, multiple sclerosis and motorneuron disease.

S26-04

LXRbeta deficiency attenuates neuroinflammation

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Liver-X-receptors (LXRs) are master regulators of cholesterol turnover and of inflammatory responses. Whereas LXRa is mainly expressed in liver, adipocytes, and immune cells, LXRb is ubiquitously expressed, being the most prominent isoform in the CNS. Recent findings demonstrated that pan-LXR agonists ameliorate the disease course of experimental autoimmune encephalomyelitis (EAE), the animal model for inflammatory demyelination in multiple sclerosis (MS). Moreover, we previously reported that activation of LXRs by myelin induces a beneficial phenotype in macrophages by suppressing pro-inflammatory mediator production. Here, we determined the contribution of endogenous LXR activation to a neuroinflammatory response by inducing EAE in mice deficient for the LXRalpha or the LXRbeta isoform. Surprisingly, in mice deficient for LXRbeta, disease severity and neuroinflammation were strongly reduced in the early disease stages, which was not seen in LXRalpha deficient mice. This indicates that LXRbeta activation can promote neuroinflammation. LXRbeta deficient mice subjected to EAE display less immune cell infiltration as well as a reduced chemokine production in the CNS. Moreover, this phenotype was independent of LXRbeta expression in peripheral immune cells. In line with these findings, an LXR agonist induced the expression of chemokines in a microglia, pointing towards a prominent role for microglia in the effect LXRbeta has on neuroinflammation. These findings indicate that endogenous LXRbeta activation controls immune cell infiltration into the CNS by inducing chemokine expression in microglia.

SYMPOSIUM 27
Oligodendrocyte metabolism and global brain health: a forgotten story back in the spotlight

S27-01

Oligodendrolglia in neurodegeneration: unexpected glial death and major role in metabolic support of neurons

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Oligodendroglia are the myelinating cells of the CNS. In addition to this function, studies of human diseases and animal models suggest that oligodendroglia play a supportive role for axons (and neurons). The exact nature of this supportive role was not clear until recent evidence demonstrated that oligodendroglia provide critical metabolic support to axons in the form of monocarboxylates (i.e., lactate). Alterations of the monocarboxylate transporter (MCT) on oligodendroglia (i.e., MCT1) produce axon and neuron degeneration. This transporter also appears to be important for human disease since MCT1 is downregulated in motor cortex of amyotrophic lateral sclerosis (ALS) patients and spinal cord of a mouse model of ALS. Additionally, protecting oligodendroglia in this model of ALS, by removing the mutant gene specifically from oligodendroglia, delays onset and prolongs survival, but also attenuates the loss of MCT1. Despite these advances, little is known about the source of oligodendroglial lactate, if cellular linkage between glia is essential for its transport, which MCT is utilized for importing lactate into axons, or whether astrocytes or other molecules are also important for the function of MCT1 in ALS. Furthermore, there is accumulating data that the oligodendroglia themselves are injured in ALS. This presentation will review recent advances in understanding oligodendroglial injury in neurodegeneration and the novel metabolic pathways by which oligodendroglia and astroglia support neurons. We hypothesize that lactate supplied to axons through MCT1 in oligodendroglia and MCT2 in axons is dependent on astrocytic interglial connections.

S27-02

Energy deprivation alters the node of Ranvier on myelinated axons

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In myelinated axons, the length and diameter of the node of Ranvier need to be controlled as the speed of Nav channel activation is partly determined by the capacitance of the node and hence its membrane area. It has been reported that the length of the node of Ranvier is increased when the energy supply is reduced in ischaemia. Here, we have investigated the mechanisms underlying changes at the node of Ranvier which occur in an experimental model of ischaemia. We find that, following inhibition of ATP production by glycolysis and oxidative phosphorylation, the node of Ranvier increases in length approximately 2-fold. This effect is dependent on Nav channel activation and an influx of calcium mediated by reversal of the Na^+/Ca^{2+} exchanger. This node elongation occurs without demyelination or a change in the localisation of Kv channels. Our results suggest that early changes in the node of Ranvier could result in altered action potential propagation independent of demyelination.

S27-03

Oligodendroglial support of axonal energy metabolism

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Metabolic cooperation between different types of brain cells has been a major topic of research in brain energy metabolism for many years. While maintenance of the energetics of action potential propagation is a major function of metabolism in white matter, oligodendrocytes have only recently received more detailed attention in respect to their metabolic interaction with axons primed by some pioneering discoveries. For example, oligodendrocytes lacking functional mitochondria are still able to support the axons enwrapped by their myelin sheaths, most likely via release of metabolites via MCT1. However, the precise pathways of metabolic support of oligodendrocytes to axons as well as its regulation and physiological impact are still to be elucidated. To address the metabolic dynamics of these interactions, we used FRET based sensors to image metabolites over time. Activation of NMDA-receptors on primary oligodendrocytes induced a rise in intracellular glucose due to increased uptake. Taking advantage of an oligodendrocyte specific NR1 knockout, we provide strong evidence that this regulation by NMDA receptors of glucose uptake into oligodendrocytes is crucial for setting up the hardware of metabolic axo-glia interaction in vivo. Furthermore, ex vivo optic nerve preparations of transgenic mice expressing a fluorescent ATP sensor in axons were used to simultaneously perform electrophysiological recordings of compound action potentials and imaging of metabolites in axons of both wild type and myelin mutant mice. We found that ATP content in axons is indeed subject to fast and reversible changes during physiological activity, which are modulated by neighbouring oligodendrocytes. In summary, we 1) describe a first non-pathological, but physiological function of NMDA-receptors on oligodendrocytes; 2) present insights in the coordinated metabolism of axons and oligodendrocytes by directly looking at the relevant molecules, the metabolites, themselves.

S27-04

The function of proteolipid protein in mitochondria: the cell's weapon for murder and self-destruction

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The main role of oligodendrocytes (Olgs) that comes to mind is the formation and maintenance of myelin. The absence of myelin in humans is associated with white matter neurodegenerative diseases in children and MS in adults. In the 70's and 80's, though, Olgs were described as having high rates of metabolism, supporting in some unknown manner the well-being of neurons. This symposium revisits the theme of Olg metabolism and how it, independently of myelin, affects the viability of neurons and stability of myelin. In this line of thinking, proteolipid protein (PLP) has been mainly viewed as a structural component of myelin, as mutations of the *PLP1*/*Plp1* gene lead to demyelination and the basis for Pelizaeus-Merzbacher disease. But mutations of the *PLP1*/*Plp1* gene have revealed novel functions that include insertion of PLP into human and rodent Olg mitochondria when the wild-type (wt)

gene is duplicated, (Hüttemann et al., 2009; Appikatla et al., 2014) with disastrous metabolic consequences. ATP levels and mitochondrial membrane potential are dramatically reduced, pH of culture media and brain ECF are sharply acidified (partially due to increased lactate), amongst other abnormalities. Insertion of PLP into mitochondria is not happenstance but regulated by the Mia40/Erv1 mitochondrial insertion pathway that requires Cx3C and Cx9C cysteines in the target protein. By mutating these specific cysteines but not cysteines in wtPLP or by pharmacologically blocking the Mia40/Erv1 insertion pathway, we prevent abnormal metabolism. It may seem evolutionarily peculiar that wtPLP should be targeted to mitochondria only when the gene is duplicated. Indeed, in normal mice co-localization of PLP is exceedingly rare but often occurs in human diseases and their models. In the human disease periventricular leukomalacia and in hypoxic-ischemic (HI) mice, we now find that wtPLP is transported into Olg mitochondria. When HI is induced in PLP knock-out and wt-mice, only wt-mice show infarcts, suggesting PLP is causative in inducing Olg death and demyelination. Likewise, we find PLP is inserted into Olg mitochondria of EAE mice. In both models, Olgs are dying. Downstream of PLP's insertion into mitochondria, we show mitochondrial molecules leak into the cytoplasm and/or into the ECS to activate an apoptotic cascade in Olgs. Our preliminary data strongly suggests that PLP functions as an intrinsic integrator of different extrinsic apoptotic signals to shift its transport away from myelin and into mitochondria where it functions as an activator of apoptosis.

SYMPOSIUM 28
Aging-related changes and their effects on astrocytes in the healthy and damaged CNS

S28-01

Astroglial complex senescent phenotype and its contribution to neurodegeneration

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Neuroglial cells are fundamental for brain homeostasis and therefore represent the intrinsic brain defence system. Thus, ageing and all forms of neuropathological processes inevitably involve glial cells. Neurodegenerative diseases, including Alzheimer´s disease (AD) disrupt brain connectivity affecting neuronal-neuronal, neuronal-glial and glial-glial interaction. Furthermore, neurodegenerative processes trigger universal and conserved glial reactions classically, but not exclusive, represented by astrogliosis and microglial activation. The recently acquired knowledge allows us to regard ageing and neurodegenerative diseases as primarily gliodegenerative processes, in which glial cells differentially determine the progression and outcome of neuropathological processes such as ageing and AD among others. We have recently probed this active pathological role, by showing: (i) an astroglial generalised atrophy with a concomitant astrogliosis just restricted to Ab plaques presence in the case of AD, whilst global hypertrophic behaviour in ageing processes, except in the entorhinal cortex, ii) alterations in glutamate glial metabolism and changes in S-100b trophic factor in both ageing and AD and finally (iii) an early resting microglial recruitment in AD affected areas, even before the presence of activated/macrophagic microglial cells. These glial alterations, which are complex and region dependent are fundamental for the disruption of neural networks connectivity as well as with the neurotransmitters imbalance that underlie the mnesic deficits associated with AD and ageing, even if they show a differential pattern and behaviour in both processes. All this, could trigger new insights into the search for a potential therapeutic treatments for pathological ageing and AD.

S28-02

Astrocytes in the aging brain and in Alzheimer's disease

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Astrocytes are actively involved in neuronal communication. In age-related neurodegenerative diseases, such as Alzheimer's disease (AD), astrocytes become reactive. The research in my lab is

focused on understanding the molecular and functional changes in reactive astrocytes, and the consequences for neuronal communication. Whole genome-wide analysis of acutely isolated astrocytes from aged and AD mice revealed that aged astrocytes have a more immune activated phenotype [1] and that the neuron-support function of AD astrocytes is declined [2]. We also observed that reactive glia in AD have an increased expression of the immunoproteasome subunits [2], resulting in an increase in immunoproteasome activity in AD brains [3]. In our current studies we are focusing on the functional changes in reactive astrocytes and their effect on neuronal communication in models for brain disease.

Reactive astrocytes have a more pronounced intermediate filament network, and the characteristic protein in this cytoskeletal network is Glial Fibrillary Acidic Protein (GFAP) [4]. Absence of GFAP in AD mouse astrocytes leads to a slightly more inflammatory phenotype [5]. Modulation of the GFAP network in an astrocytic cell line, results in significant changes in genes involved in cell-cell signaling and interacting with the extracellular matrix [6, 7]. Taken together, our data show that a change in the GFAP intermediate filament network can change the transcriptome of astrocytes, thereby potentially adapting physiological processes in these cells.

In conclusion, our research shows molecular changes in astrocytes due to aging and degenerative processes. These changes might be partly induced by the modulation of the GFAP constitution of the astrocytic intermediate filament cytoskeleton.

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S28-03

Age-related alterations of proliferative capacity and the stem cell potential in reactive astrocytes

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Astrocytes play the central role in brain homeostatic system, ensuring optimal neuronal functioning through their multiple functions. Nowadays an increasing body of evidence suggests that astrocytes are also specifically involved in structural and functional brain changes associated with normal ageing. However, as astrocytes are highly heterogeneous in their morphological and physiological features, it is not surprisingly that they may react to the aging process in a different manner. In view of this heterogeneity, aging seems to be associated with progressive increase of gliosis-like changes in astrocytes, even when no sign of disease is apparent. These include hypertrophy, elevated expression of cytoplasmic antigens and surface proteins, as well as secretion of growth factors. Thus, if astrocytes occur such a complex remodeling during physiological aging, then to which extent this could have an influence on their response to pathological conditions in the aging brain. As an invasive injury can induce plasticity in a subset of reactive astrocytes, we investigated whether and how proliferative properties in the cortical reactive astrocytes change with aging. Our observations provide not only new insights into the age-related alterations of both proliferation and stem cell capacity of astrocytes reacting to invasive injury in the cerebral cortex, but also uncover aging effects of the mode and

multitude of reactive astrocyte cell divisions, open therefore new paths for a better understanding of the cellular mechanisms by which age contributes to progressive losses of the injury-mediated plasticity in the brain.

S28-04

Phenotypic plasticity of astrocytes in aging and disease

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CD44, a plasma membrane protein and extracellular matrix receptor, characterizes long-process/interlaminar astrocytes in the human CNS. These astrocytes, which give rise to long, unbranched proceses, reside in subpial regions, white matter, and adjacent to large blood vessels. CD44+ astrocytes generally display high immunostaining for GFAP, S100β, SPARC, and αB-crystallin, but low immuno-staining for glutamine synthetase, EAAT1, and EAAT2. Thus, they conform to the so-called “fibrous” astrocyte” phenotype. CD44+ long-process astrocytes appear during fetal development and remain throughout life. In contrast, most astrocytes of CNS gray matter exhibit a protoplasmic phenotype, with well-delineated domains and highly branched processes. However, CD44+, long-process astrocytes appear in gray matter with aging and in a number of CNS disorders. We believe that these gray matter astrocytes are derived from CD44-negative, protoplasmic astrocytes, indicating that protoplasmic astrocytes will markedly alter their properties in disease.We have examined a number of neurological diseases for this astrocyte pathological transition, and have found such changes in, to name a few, Alzheimer disease, diffuse Lewy body disease, Huntington disease, varieties of epilepsy, tuberous sclerosis and cortical dysplasias, hypoxic/ischemic disease, and Alexander disease. We have reported similar changes in astrocyte phenotype in the mouse CNS in pathology, although rodent astrocytes do not assume the long-process morphology of human astrocytes. A prominent characteristic of this phenotypic change is that it displays a mosaic appearance at a microscopic level. Thus, CD44+ astrocytes are found adjacent to and intermixed with CD44-negative astrocytes, suggesting that astrocyte transitions can be regulated by highly local environmental signals or cues. The nature of those signals awaits discovery.

SYMPOSIUM 29
Enteric glia: novel roles in enteric neural circuitry and gut pathophysiology

S29-01

Emerging roles for enteric glia in the gastrointestinal tract

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The enteric nervous system (ENS) provides local neural control of the gastrointestinal (GI) tract. In particular, the ENS is necessary for the elegant coordination between the digestive and defensive functions of the gut, involving the many cellular components that are found in the gut wall. The neurons of the ENS are arranged in two ganglionated plexuses, myenteric and submucosal, that extend along the entire length of the gut. The neuropil of enteric ganglia is similar to the brain, consisting of neurons that are surrounded and slightly outnumbered by enteric glial cells. These cells resemble astrocytes of the CNS and are not only found in enteric ganglia, but also in the mucosa of the GI tract, lying just beneath the epithelium. It has become abundantly clear that enteric glia are key players in gut physiology and pathophysiology; including the control of intestinal motility, the maintenance of intestinal barrier function, the regulation of inflammation and as a primary target for neurotropic viruses and prions. Selective ablation of enteric glia in transgenic mice results in severe intestinal inflammation, neuronal degeneration and ultimately death, indicating a vital role for enteric glia in maintaining the integrity of the ENS and, in turn, normal gut function.

Whilst initially regarded as passive support cells, it is now clear that enteric glia are actively involved as cellular integrators in the control of motility and epithelial barrier function. Enteric glia form a cellular and molecular bridge between enteric nerves, enteroendocrine cells, immune cells and epithelial cells depending on their location. This presentation will highlight the role of enteric glia in purinergic signaling in the ENS and barrier and defense functions of the gut, notably in states of inflammation. We have recently demonstrated a novel role for enteric glia located in the ENS in the dysregulation of intestinal ion transport during colitis. Enteric glial-derived nitric oxide appears to alter signaling in the ENS resulting in reduced electrogenic secretion. This has functional consequences on the integrity of the epithelial barrier, and, as a result, on the severity of colitis. From a clinical standpoint, enteric glia represent new and potentially important targets for drug treatment in disorders of motility and host defense, notably in inflammatory conditions of the gut.

S29-02

Development and homeostasis of enteric glial cells

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Enteric glial cells (EGCs) are essential for enteric nervous system (ENS) function and gastrointestinal homeostasis. Using Mosaic Analysis with Double Markers (MADM) we have previously identified four morphologically distinct subpopulations of EGCs in the mouse gastrointestinal tract which can be distinguished by the combinatorial expression peripheral glial markers, such as glial fibrillary acidic protein (GFAP), S100β, and Sox10. Moreover, using Ca(2+) imaging we have shown that extraganglionic glia manifest a differential response to adenosine triphosphate (ATP) relative to intraganglionic type I EGCs. Lineage tracing studies have also suggested that the subpopulations of EGCs are highly dynamic and capable of responding to environmental factors. This is highlighted by the postnatal development of mucosal glial cells and the continuous renewal of this cell population by incoming glia originating in the outer myenteric plexus. Interestingly, both the postnatal development and the homeostatic behavior of mucosal EGCS in mice are controlled by the luminal microbiota. We propose that the supply of new mucosal EGC progenitors depends on the activation (A) of ganglionic glia which normally remain in a quiescent (Q) differentiated state but can be activated by as yet unknown signals. Here we will report on experiments that explore the extrinsic signals and the cell-intrinsic mechanisms that underpin the Q to A transition of adult EGCs. Our studies have important implications for the understanding of the mechanisms that regulate the development and homeostasis of glial cells in mammalian organisms.

S29-03

Glial functions in enteric neural circuits

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The enteric nervous system (ENS) is a crucial ganglionated nerve network embedded in the mucosal and muscular layers of the entire intestine. From there it controls many of the gastrointestinal functions including motility, absorption and secretion. Apart from different types of neurons, the ENS also consist of numerous glial cells, which were originally thought to be fairly simple cells that provide structural and trophic support for the adjacent neurons. In the last couple of decades it has become apparent that, as in the CNS, enteric glial cells play more prominent roles than originally attributed. Enteric glia have shown to be essential for gut homeostasis, to mediate inflammatory events and even to support host defense. Apart from these rather established functions, little is known about the role of glial cells during the physiological tasks of the enteric nervous system. What role do they play when functional circuitry is being formed, how do they influence enteric synaptic transmission and activity propagation? In this presentation the current knowledge of the different types of enteric glial cells with respect to nerve circuitry function will be reviewed. There will be a strong focus on live imaging as well as on genetic and immunohistochemical tools to specifically label and study enteric glial cells. The advantages and limitations of microscopy and imaging techniques for studying enteric glia function will be discussed both with respect to their use in experimental animal models as well as in human intestinal biopsies.

S29-04

Interactions between enteric glia and neurons in disease

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The concept of enteric glia as key regulators of intestinal homeostasis is slowly gaining acceptance as a central concept in neurogastroenterology. Yet how glia contribute to intestinal disease is still poorly understood. One popular hypothesis is that the loss of glial supportive functions contributes to enteric neuropathy because enteric neuron death is observed in models of glial ablation. However, new data show that chronic astroglial activation, rather than glial cell loss, is responsible for driving neurodegeneration during neuroinflammation in the brain. We recently discovered that enteric glia are activated by purines released from enteric neurons prior to neuronal death during colitis. Specifically, the activation of neuronal P2X7 purine receptors (P2X7Rs) triggers the release of ATP from neurons through pannexin-1 (panx1) channels as a signal to enteric glia. In the brain, neuronal ATP release through panx1 is considered a danger signal that glial cells interpret as a "search and destroy" message; causing glia to execute otherwise healthy neurons. Given that stimulation of P2Y1Rs is a potent stimulus for reactive astrogliosis in the central nervous system, we hypothesized that the activation of glial purine receptors contributes to neuropathy in the ENS. We tested our hypothesis using a combination of *in vivo* models of colitis with inducible and conditional transgenic mice and *ex vivo* intestinal preparations to address specific mechanisms. Our data show that glial activation is sufficient to cause enteric neuron death via a mechanism that depends on the activation of connexin-43 (Cx43) hemichannels and subsequent ATP release. Surprisingly, our data show that glial pathways dependent upon intracellular Ca^{2+} signaling are not sufficient to drive neuronal death. Instead, glial-driven neuron death requires the gating of glial Cx43 hemichannels by nitric oxide (NO). In all, our results show that the activation of glial cells in the context of neuroinflammation kills enteric neurons. Mediators of inflammation that include ATP and NO activate neurotoxic pathways that converge on glial Cx43 hemichannels. We propose that the glial response to inflammatory mediators might contribute to the development of motility disorders.

SYMPOSIUM 30
A disrupted gliogenic/neurogenic equilibrium is essential for the injury response of adult neural stem cells

S30-01

Reactive neural stem cells in the adult hippocampus

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Radial neural stem cells (rNSCs) dwelling in the dentate gyrus of the hippocampus generate new neurons throughout adulthood. The population of rNSCs loses their mitotic capabilities and declines progressively over time in part due to their activation-dependent conversion into astrocytes. We hypothesized that overactivation of rNSCs by neuronal hyperexcitation could hasten rNSC depletion population and accelerate the age-associated decline of neurogenesis. Using a mouse model of mesial temporal lobe epilepsy (MTLE), we show that seizures induce rNSCs to acquire a reactive phenotype and to enter a symmetrical mode of mitosis in which both mother and daughter cells differentiate into reactive astrocytes. Furthermore, epileptiform activity (EA), neuronal hyperexcitation

without seizures, provokes an increase in rNSC activation and subsequent depletion, speeds up the decline of neurogenesis. Our results demonstrate that abnormal levels of neuronal activity provoke a dramatic shift in rNSCs function, which can impair adult hippocampal neurogenesis in the long term. We are investigating whether blocking rNSCs activation can preserve the rNSC pool and neurogenesis in MTLE. In addition, we have observed that during aging, those rNSCs remaining in the dentate gyrus also acquire a reactive phenotype. Our results show new properties of hippocampal rNSCs that could convert them in the target of therapeutic strategies to fight against neurological diseases such as epilepsy, and aging.

S30-02

Glio- and neurogenesis after irradiation: implications for hippocampal function

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New dentate gyrus hippocampal neurons - and thus by extension the radial glial-like cells from which they emerge - have been implicated in a variety of functions, including contextual discrimination, mood control, and addiction. Here I will address three unanswered questions relevant to the gliogenesis, neurogenesis, and hippocampal function by highlighting published and unpublished work from my laboratory. First, are all adult-generated dentate gyrus neurons from nestin-lineage radial glial cells? Second, do nestin-lineage radial glial cells respond to physiological stimuli that increase (e.g. running) or decrease (e.g. X-ray irradiation) neurogenesis? Finally, is the number of adult-generated neurons always associated with improved context discrimination? For this latter point, we will highlight our unpublished, NASA-funded work examining the age-dependent influence of galactic cosmic radiation on hippocampal function and cellular composition. I will end by discussing the emergence of network level approaches to manipulate neurogenesis and hippocampal function in laboratory animals, and comment on the future of such approaches for translational application, particularly outside the scope of early life and into maturity.

S30-03

Molecular heterogeneity of adult neural stem cells

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Neural stem cells (NSCs) are essential for brain maintenance and regeneration after injury. In the adult subventricular zone, NSCs generate several types of neurons to maintain cellular homeostasis in the olfactory bulb and can participate in cell replacement after brain injury. It is assumed that the balance between quiescence and activation of NSCs and the early induction of specific transcription factors programs regulate the number and diversity of their progeny . However, the molecular hallmarks of NSC activation and specification have remained unclear in part due to the limitations of cell-population-based studies. Here, we sequenced the transcriptome of single NSCs isolated from their in vivo niche in the subventricular zone during homeostasis as well as following ischemic injury. In homeostasis, we identify a previously unrecognized dormant subpopulation of NSCs that is distinct from mature parenchymal astrocytes, and can be identified with novel markers. These cells already express transcription factor combinations indicative of the lineage bias. During lineage progression, dormant NSCs transition to a primed-quiescent state through upregulation of the protein synthesis machinery. These cells later proceed to activation via repression of Notch and Bmp and induction of the differentiation program. During brain repair, dormant NSCs rapidly shift to the primed-quiescent in response to injury-induced interferon gamma (IFN-γ). This study provides general molecular principles of neural stem cell activation during brain homeostasis and repair and opens new avenues for regenerative medicine.

S30-04

Identity and function of neural stem cells in the spinal cord

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The neural stem cells in the adult spinal cord are ependymal cells. After spinal cord injury, ependymal cells are activated and produce more than half of the astrocytes in the glial scar forming at the injury site, and a small amount of remyelinating oligodendrocytes. Ependymal cell contribution to the glial scar is essential to regain tissue integrity and limits neuronal loss after spinal cord injury. These functions highlight ependymal cells as interesting targets for altering the cellular response to spinal cord injury to preserve or regain function.

Ependymal cells are heterogeneous in morphology and protein expression, but there is limited evidence of functional heterogeneity and no previous reports of ependymal cell subpopulations with neural stem cell properties. Pinpointing the exact identity of the neural stem cells within the ependymal cell population is important to develop therapies that modulate the endogenous neural stem cell response to spinal cord injury.

We have assessed stem cell properties of functionally separate subpopulations of ependymal cells with genetic fate mapping. We show that the neural stem cell potential of the adult spinal cord is contained within a small subpopulation of ependymal cells, and that another ependymal cell subpopulation contains restricted progenitor cells. Ependymal cells with stem cell properties generate both scar-forming astrocytes and oligodendrocytes in response to spinal cord inury, and could pose an interesting target for modulating the endogenous neural stem cell response to spinal cord injury.