Angiogenesis in the brain – from health to disease

Neuro-vascular communication during CNS development

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The formation of a precise vascular network within the central nervous system (CNS) is of critical importance not only to assure proper delivery of oxygen and nutrients but also for accurate functionality of neuronal networks. As dysfunction of neuronal networks is a hallmark in any neurological disease, understanding how those networks are formed in correlation with the other neighboring cells of their environment is crucial for proper neuroprotection and neurorepair.

The CNS is initially avascular and becomes vascularized by blood vessel sprouting from a perineural vascular plexus. Although neuronal-derived Vascular Endothelial Growth Factor (VEGF) is crucial for CNS vascularization, the signaling transduction pathways that result into an angiogenic response are only partially understood. Here we will discuss how VEGF uses the co-transcription factors YAP/TAZ to exert this effect.

Despite the important function of VEGF for vessel growth, blood vessel patterning in the CNS cannot be explained by the sole presence of VEGF. Vascularization of the spinal cord is a highly stereotypical process. In this talk, we will discuss evidences for a motor neuron specific autocrine mechanism that tightly regulates the availability of pro- and anti-angiogenic guidance factors to control spinal cord vascularization and thus to guarantee proper blood vessel patterning.

Angiogenesis in small vessel disease

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Cerebral small vessel diseases lead to a rarefaction of vessels in the CNS. The question whether angiogenesis is able to compensate for the loss of vessels and its functional consequences is largely unknown. To investigate this issue, we used *NemobeKO* mice as an inducible model of small vessel disease. The mice are deficient of the NF-κB essential modulator (NEMO) and mimic the human disease incontinentia pigmenti (IP) which manifests with severe neurological symptoms. Previous work has shown that, after inducing recombination by administration of tamoxifen, *NemobeKO* animals develop a disturbed blood-brain barrier (BBB) and a loss of cerebral capillaries. Here, we found proliferation of endothelial cells and other cells in *NemobeKO* mice. Angiogenesis peaked on day 15 after inducing the knock-out and was spatially associated with apoptosis, increased vessel death and hypoxia. In parallel, body weight, mortality rate, locomotor activity and anxiety-like behavior normalized suggesting that angiogenesis could promote functional recovery. To test this hypothesis, we continued tamoxifen treatment for several weeks and thereby compromised the survival of new capillaries. Interestingly, low survival of new capillaries impaired the functional recovery of mice. Over time, the animals developed cognitive deficits. In summary, our data suggest that angiogenesis may protect against some aspects of small vessel diseases and may be a suitable target for their treatment.

Principles of vascular adaptation – an endothelial cell perspective

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How endothelial cells shape and maintain a functional hierarchically branched vessel network to supply all organs in physiology and orchestrate a regulated response to adapt this network to changing hemodynamic and metabolic requirements is one of the most fascinating fundamental questions in vascular biology and medicine. Combining cell biology approaches in vivo in mouse and zebrafish with flow rheology models and generative computational models, as well as mosaic genetic approaches, we identified an astonishing degree of endothelial cell dynamics that underlie the key processes of vessel formation and adaptation. Whereas the initial sprouting and branching of new vessels is regulated by feedbacks between tissue derived vascular growth signals and endothelial cell-cell communication to establish a balance of the right numbers of sprouting cells and proliferating, tube forming cells, as well as their distribution, the subsequent remodelling of the network is governed by endothelial responses to changing blood flow and the resulting shear forces. This presentation will provide a short overview of the central concepts and molecular regulators at play and give an outlook into possible implications for vascular disease and maladaptive responses.

Protective strategies for intracerebral hemorrhage-induced brain damage

Dynamic changes in immune responses early after ICH

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Inflammatory responses after injury rapidly shift from pathogen defense to tissue repair. Mounting evidence suggests these paradigms also apply to acute sterile injuries within the brain such as ICH. As highly plastic cells, macrophages and microglia can have dynamic phenotypic changes and may direct inflammatory responses that lead to neurological injury or recovery. This presentation will discuss the time course of phenotypic changes in these two cell populations and directly compare the responses of these cellular "cousins".

Ferroptosis after intracerebral hemorrhage

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Intracerebral hemorrhage (ICH) causes high mortality and morbidity, but our knowledge of post-ICH neuronal death and related mechanisms is limited. We demonstrated that ferroptosis, a newly identified form of cell death, occurs in the collagenase-induced ICH model in mice. We found that administration of ferrostatin-1, a specific inhibitor of ferroptosis, prevented neuronal death induced by hemoglobin in organotypic hippocampal slice cultures (OHSCs). Mice treated with ferrostatin-1 after ICH exhibited marked brain protection and improved neurologic function. Additionally, we found that ferrostatin-1 reduced lipid reactive oxygen species production and attenuated the increased expression level of *PTGS2* and its gene product cyclooxygenase-2 *ex vivo* and *in vivo*. Moreover, ferrostatin-1 in combination with other inhibitors that target different forms of cell death prevented hemoglobin-induced cell death in OHSCs and human induced pluripotent stem cell–derived neurons better than any inhibitor alone. These results indicate that ferroptosis contributes to neuronal death after ICH, that administration of ferrostatin-1 protects hemorrhagic brain, and that cyclooxygenase-2 could be a biomarker of ferroptosis. The

insights gained from this study will advance our knowledge of the post-ICH cell death cascade and be essential for future preclinical studies.

Novel strategy to limit hematoma growth following intracerebral hemorrhage.

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Spontaneous intracerebral hemorrhage (sICH) is the deadliest stroke sub-type. No effective treatment is available so far. Preventing hematoma extension, and/or prevention of continued bleeding in sICH are attractive therapeutic targets. It has been shown *in vitro* that red blood cell-derived microparticles (RMP) enhance platelet function and accelerate coagulation, augmenting both primary and secondary hemostasis. In the present study, we determined the efficacy of RMP in reducing hematoma expansion in a rat model of sICH. We will present our recent results on the efficacy of RMP in preventing hematoma growth in a rat model of collagenase-induced sICH. Support: NIH grant NS094896.

Recent advances in neurorehabilitation strategies in stroke

Novel approaches in experimental neurorehabilitation

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Ischemic injuries within the motor cortex results in functional deficits that profoundly impact activities of daily living in patients. Current rehabilitation protocols achieve only limited recovery of motor abilities. The brain reorganizes spontaneously after injury, and it is believed that appropriately boosting these neuroplastic processes may restore function via recruitment of spared areas and pathways. In this presentation, I will describe our recent work on novel experimental therapies for the recovery of motor function in a mouse model of focal stroke. Specifically, we have tested experimental approaches in which physiotherapy is coupled with delivery of plasticising drugs that render the spared, undamaged pathways more sensitive to experience-dependent modifications. These combinatorial strategies hold promise for the definition of more effective rehabilitation paradigms that can be translated into clinical practice.

Rehabilitation and cell-based therapies in stroke

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Stroke is a leading cause of adult disability. Recent advances in acute stroke care has meant that more and more patients survive, but are left with permanent impairments. Less than 10% of stroke patients receive thrombolysis or mechanical thrombectomy due to their narrow treatment window. Thus, novel therapies beyond acute care are urgently needed. The major advantage of restorative strategy as compared to acute treatments is its wider therapeutic time window, which means that interventions would be available for a larger percentage of stroke patients allowing also the combination of different therapies. Much hope is placed on cell therapy in stroke, however, early phase clinical trials have shown only minimal treatment effects far from optimal. Heterogeneity of stroke patients including variable content of rehabilitation may contribute to low treatment efficacy. Experimental evidence suggest that cell therapy might open brain plasticity which is then further enhanced by rehabilitation. Most likely multiple, intermingling mechanisms are involved leading to improved behavioral outcome. However, a major challenge is to discriminate stand-alone therapeutic effects from add-on therapy effects in small preclinical studies.

Developing novel adjunct therapies post-stroke; lessons from neuroimaging and neuromodulation

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Non-invasive brain stimulation (NIBS) approaches offer significant potential as putative adjunct therapies for stroke, but their use is currently limited by the variability of their effects. Therefore, before the full potential of NIBS can be determined it is necessary to understand more about the mechanism of action. The effects of NIBS occur across multiple spatial and temporal scales; from the synapse to the network and from effects lasting seconds to those lasting months or years. To attempt to understand this diversity of effects, advanced neuroimaging approaches, including MR Imaging, MR Spectroscopy and electrophysiological methods such as magnetoencephalography (MEG) and electroencephalography (EEG) have been combined with NIBS. These approaches have revealed many important underlying mechanisms of NIBS, but they also have their limitations, and more needs to be done, particularly in combining multimodal imaging with NIBS, before the full utility of neuroimaging to understand the neurophysiological underpinnings of NIBS can be revealed.

Here, I will discuss recent studies from our group using NIBS to study the physiological basis of motor plasticity *in vivo*, in combination with MR Imaging, MR Spectroscopy and Magnetoencephalography. These studies provide increasing, converging evidence that changes in local and network-level inhibitory processing is a key component of motor learning. I will highlight how inter-individual differences may prove important for predicting response to potential interventions post-stroke.

Towards precision medicine: patient-tailored treatment strategies to enhance motor recovery after stroke

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In Europe, 3.7 million patients suffer from long-term deficits, such as motor or language deficits, after a stroke. Despite the recent developments in acute stroke therapy (e.g., thrombolysis, thrombectomy, stroke units) still less than 15% of the patients recover to a degree that they get back to normal life. This makes stroke the main course of long-term disability with major impact on patients" professional and private life, the health systems and socio-economics. Thus, there is a strong need for novel, innovative treatment strategies to enhance significantly the magnitude of functional recovery to bring more patients back to normal life.

Innovative treatment strategies, such as non-invasive brain stimulation (NIBS), robot-, VR- or BCI-based treatments, have demonstrated promising results in proof-of-principle studies (Hummel et al., 2005, Hummel &Cohen, 2006, for review Raffin & Hummel, 2017). However, the treatment responses are not satisfying yet, as their magnitude is heterogeneous, with responders and non-responders. For example, one reason for this might be that these neurotechnology-based interventions are used in non-precision "*one suits all*" approaches independent of the characteristics and requirements of the individual patients. Based on the fact that the population of stroke patients is quite heterogeneous in relation to e.g., lesion location, lesion size, course of recovery, initial deficit, functional and structural pre-requisites beyond others, "one suits all" seems not to be the most promising approach (Schulz et al. 2015, 2017, for review Koch & Hummel, 2017, Wessel &Hummel, 2017).

Thus, to achieve treatment effects with much larger magnitude, there might be a need for a paradigm shift from imprecision "one suits all" treatment strategies towards patient-tailored precision medicine approaches. In the present talk, these issues will be discussed in more detail and potential approaches towards patient-tailored interventions to achieve homogenous treatment responses with maximized effects will be introduced.

Next generation functional testing in experimental brain research

New avenues in rehabilitative training and functional testing in rodent models of cervical spinal cord injury

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Question: Rehabilitative training is standard following injuries of the nervous system, and currently the most efficient treatment for individuals suffering from spinal cord or brain injury. To adopt training paradigms at sufficient intensity in rodent models is difficult and time consuming. Consequently, the knowledge about optimal training paradigms and the interaction between training and other treatments is underwhelming. Over the last years we addressed the question of how training in rodent models of SCI is best administered, how it influences neuroplasticity and how it can be applied in a manageable, even automated manner.

Methods: With a focus on cervical spinal injuries we studied the interaction between training in a single pellet reaching task and recovery in adult female rats. Training intensity was increased by automating the task to even reach a fully automated training and testing environment. Various pharmacological treatments were applied to evaluate the interaction between their plasticity promoting effect and training.

Results: Rehabilitative training in rats with cervical spinal lesions significantly increases neuroplasticity and guides pharmacologically induced plasticity to functional meaningful connections. Although there is a window of opportunity for efficient training this window can be reopened with various approaches including an inflammatory stimulus. However, training needs to be applied at sufficient intensities to translate to functional recovery. Automating this process greatly facilitates the research progress by reducing variability and enabling the required training intensity.

Conclusion: Rehabilitative training should be part of any preclinical research design to truly evaluate functional recovery, which can be facilitated using automated training setups.

Animal models for psychological trauma-induced changes in brain structure and function: sex-specific vulnerability, resilience and strategies for protective interventions.

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The negative long-term consequences of early life adversities are well-recognized in mental health research, however, the cellular and molecular events underlying stress- and trauma-induced neuronal “scars” in the brain, in particular those involved in the intergenerational transmission, are not known in detail. Animal models allow to “zoom in” on the impact of early life adversities on the functional maturation of prefronto-limbic circuits using functional imaging, structural synaptic analysis and epigenetic changes. It is essential to gain a more detailed understanding of these mechanisms to develop novel individually (e.g. sex-specific) tailored protective and therapeutic interventions.

Indicators and Modulators of Functional Recovery and Compensation

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The acquisition of new motor skills critically depends on experience and practice. Experience-dependent motor skill learning represents a central mechanism by which individuals with a brain lesion overcome loss of motor function. Most individuals with a brain lesion are required to learn some kind of compensatory movement strategies over time that substitute for lost motor capacity. Our work showed that compensatory movement strategies are clearly recognizable and distinguishable from original, intact movements, and they reflect anatomical rearrangements of the underlying neuronal pathways. We will discuss methods of assessment of compensation versus recovery of function using motor skill as an exemplary model. Because compensation in the lesion brain is mediated by the same neural mechanisms as those that contribute to development and aging, the study of compensation also leads to insights into nervous system function on a more general level.

Can traumatic brain injury serve as a model to study neurovascular unit dysfunction in neurodegenerative disease?

Advanced neuroimaging techniques to characterize brain alterations following TBI and repetitive head impacts

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Neuroimaging technology has made rapid advances that have changed the way medical imaging is used in both research and in clinical settings. This presentation addresses several advances and challenges in neuroimaging from the development of new imaging techniques and sequences that may enhance the use of imaging to elucidate the underlying pathomechanisms of traumatic brain injury (TBI) and related neurodgenerative disorders, to the challenges of imaging in large-scale multisite projects of TBI, to the potential clinical applications of imaging in specific populations with TBI. More specifically, Dr. Inga Koerte will provide an overview of "state-of-the-science" in structural and diffusion imaging techniques in mild TBI and repetitive head impacts. She will then briefly review recent efforts in mTBI to standardize imaging acquisition and analyses in order to enhance "big data". Dr. Koerte will also present data from special TBI populations such as athletes who sustain repetitive sports-related head impacts and military members exposed to mTBI and blast. Finally, Dr. Koerte will review novel analytic strategies of potential utility in individualized, precision-medicine.

Neuroimaging changes at longterm after a single mild TBI in childhood: neurovascular unit dysfunction

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Traumatic brain injury (TBI) is the first cause for emergency department visits in the pediatric population with a majority of mild TBIs. Mild TBI is defined with a Glasgow coma score of 13-15, no or transient (< 30 min) loss of consciousness, no skull fracture, no visual alterations on CT scans, and no apparent short-term cognitive deficits. It is now recognized that moderate/severe jTBI causes significant morbidity over years after the initial event. There

are clinical works showing that repetitive TBI can induce cognitive dysfunctions such as chronic traumatic encephalopathy. However, much less is known about the long-term consequences of single mild jTBI on cognitive functions, brain structures and underlying molecular and cellular mechanisms. Therefore, we aimed to explore the consequences of a single juvenile mild TBI (jmTBI) in the aging process by evaluating: a) cognitive function with a battery of behavioral tests, b) neuroimaging alterations using diffusion tensor imaging (DTI) and c) histological changes (gliovascular and neuronal alterations) from day 1 to 12 months after one jmTBI sustained at postnatal day 17 in mouse.

Behavior dysfunctions emerged over time with increase of anxiety at 1 month, then significant impairment in spatial learning and memory at 12months after a single jmTBI event. These cognitive impairments were accompanied by DTI changes in both white and grey matter: decreased fractional anisotropy (FA) was observed in the corpus callosum and most parts of the dorsal and ventral hippocampus. In addition, a significant decrease of AD was observed in the substantia innominata/nucleus basalis at 12 months, a region that has also been involved in cognition. Interestingly, early phenotypic changes in neurovascular unit have been observed with increase of GFAP and AQP4 expression before loss of NF200 at 1 month post-jmTBI. Then, landmarks of accelerated aging were observed at 12 months: there was a significant decrease in NeuN staining in jmTBI compared to sham group in the dorsal hippocampus. Altered neurovascular unit accompanied this with changes in astrocyte phenotype (increased AQP4 water channel in astrocyte perivascular end-feet and processes), concomitant with a reduction in blood vessel diameter.

Our work shows for the first time that a single mild TBI during the juvenile period exacerbates an aging phenotype (cognitive impairment, white and grey matter DTI alterations, hippocampal neurodegeneration) 12 months after the injury, accompanied by significant neurovascular alterations dependent of the brain regions and time after the injury. The neurovascular unit could represent a target for future treatment development.

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Progressive cognitive decline after traumatic brain injury in adult mice

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Traumatic brain injury (TBI) is a major cause of death and disability worldwide. In past decades, most research focused on clarifying the mechanisms of acute brain damage. There is, however, increasing evidence that TBI pathology may continue for years after the initial insult causing progressive histopathological changes. This chronic posttraumatic brain damage has been linked to long term functional deficits, including progressive neurocognitive decline and psychological abnormalities, e.g. dementia and depression. The aim of the current talk is to characterize long-term functional and histopathological outcome after experimental TBI in order to evaluate if previously used models to study the pathophysiology of acute post-traumatic brain damage are also suited to study chronic sequels of TBI.

Male C57BL/6N mice (n=12 per group) underwent contusional brain injury (CCI) or sham operation and were observed for up to 12 months thereafter. Neurological function was assessed over the whole observation period using test paradigms evaluating motor function (Beam Walk test), depression like behavior (Tail Suspension test), as well as spatial learning and memory (Barnes´ Maze). Furthermore, histopathological outcome was assessed histologically.

Animals recovered well from trauma for about three months. Thereafter, however, mice developed increasing depression like behavior and a progressive deterioration of spatial learning and memory. These functional deficits, which are well known from TBI patients, were preceded by progressive brain atrophy, delayed loss of gray and white matter and hydrocephalus.

Our study demonstrates progressive histopathological and functional deterioration after TBI. Surprisingly, the progressive dynamic of this process starts with a delay of several months after trauma and seems to continue for at

least one year. The main consequences of these progressive changes are depression and loss of memory. Hence, the CCI model in mice seems to mimic some of the most prominent changes observed after TBI in humans and may therefore be a suitable tool to further investigate the pathomechanisms of chronic TBI.

Vascular repair after traumatic brain injury: involvement of Wnt pathway

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Traumatic brain injury (TBI) results in damage to the cerebral vasculature and is often associated with hemorrhage, edema, blood brain barrier disruption, blood flow abnormalities, and cell death. An important and unexplored avenue is how blood vessels undergo repair and remodeling. At present, the temporal evolution of vascular repair is unknown with little knowledge of the molecular mechanism(s) underlying vascular repair after TBI. One possible molecular pathway that may be involved in vascular repair is the Wnt/b-catenin cascade. The Wnt/β-catenin pathway promotes blood vessel formation during vascular development, but its role in vascular repair after injury is unknown.

We examined how the cerebral vessels respond over 7 days following a moderate TBI focusing what role Wnt/β-catenin signaling plays in the vascular repair process. A controlled cortical impact mouse model was used to induce a moderate TBI which lead to gross injury to the cerebral vessels. Using a novel vessel painting technique to label the cerebral vessels within the entire brain, we assessed vascular alterations at 1 and 7 days post injury (dpi). We assessed b-catenin inside blood vessels around the lesion and utilized a Wnt transgenic mouse line to evaluate Wnt gene expression. To assess the role of β-catenin in vascular repair, we utilized Lithium (GSK3 inhibitor) to enhance b-catenin expression and JW74 (tankyrase inhibitor) to inhibit b-catenin expression and evaluate their effects on vessel morphology and development of hemorrhage and edema. Lithium or JW74 were administered to mice for 6 consecutive days and then sacrificed at 7 dpi.

We report that TBI results in vascular loss at 1 dpi followed by increases in vascular structure by 7 dpi. β-catenin expression was increased in peri-lesional vessels at 1 and 7 dpi. Similarly, we found increased number of Wnt-GFP-positive vessels after TBI. Treatment with Lithium in TBI mice revealed an increase in average vessel length compared to saline treated mice. Conversely, JW74 treated mice showed a robust reduction in vessel area, branch points, and average vessel length compared to vehicle treated mice. Magnetic resonance imaging T2 and susceptibility weighted imaging of vessel painted brains at 7 dpi revealed an increase in hemorrhage and edema volumes following JW74 treatment and but a significant reduction in hemorrhage and edema volumes following Lithium treatment.

Our findings suggest that endogenous developmental programs, such as Wnt/β-catenin, likely become activated after TBI to initiate repair. Treatment regimens to enhance activation Wnt/β-catenin appear contribute to the vascular repair process after TBI and represents a potential target for future therapeutics.

Emerging role of extracellular vesicles as therapeutic agents for sudden-onset neurological disorders

Emerging potential of extracellular vesicles for treatment of traumatic brain injury

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Stem cell therapy emerges as a promising approach for treatment of traumatic brain injury (TBI) for which there is no effective treatment. Recent research indicates that the efficacy of cell therapy for brain injury is mainly attributed to effects of extracellular microvesicles including exosomes generated from stem cells rather than cell replacement. Exosomes are nanosized extracellular vesicles (40-120 nm in diameter) secreted by nearly all living cells and play a pivotal role in intercellular communication. Exosomes have been recognized as potential therapeutics as they elicit potent cellular responses in vitro and in vivo via delivery of their cargos including lipids, proteins, RNAs (microRNAs and mRNAs) and other macromolecules to recipient cells. By affecting gene regulation, miRNAs are likely to be involved in most biological processes from developmental timing to host-pathogen interactions to tumorigenesis in various organisms. To enhance therapeutic efficacy of exosomes, the content and function of exosomes can be modified in various ways. Here we discuss recent advances in the use of naïve exosomes for treatment of TBI and designer exosomes with select miRNAs bioengineered to generate potent exosomes for better therapeutic efficacy.

Exosomal CRAMP derived from cerebrospinal fluid facilitates neurological functional recovery after spinal cord injury by promoting angiogenesis and attenuating inflammation

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Introduction: Spinal cord injury is a devastating global health problem and results in irreversible sensory and motor impairments. Unfortunately, there is no treatment that reverses damage to the spinal cord. Exosomes packaged vesicles containing specific molecules can directly serve as a potential therapeutic approach for tissue repair. However, the role and mechanism of cerebrospinal fluid derived exosomes in neurological functional recovery after spinal cord injury remain unclear.

Methods and results: Here, we aim to investigate the role of exosomes derived from cerebrospinal fluid (CSF-Exos) on spinal cord injury in rat and explore the involved molecular mechanism. Exosomes derived from the cerebrospinal fluid both in the injured and normal spinal cord rat exhibited same morphology and mean diameter with approximately 98.26±8.47nm. We found that, compared with CSF-Exos from normal mice (Normal CSF-Exos), exosomes from injured mouse CSF (Injured CSF-Exos) significantly enhanced the angiogenic activities of endothelial cells including the migration of endothelial cells(EC) and tube formation, stimulated AKT phosphorylation and increased VEGF-A protein expression in the cells. Furthermore, in vivo delivery of injured CSF-Exos into injured spinal cord rat facilitated neurological functional recovery by promoting angiogenesis as evidenced by increased vessel number and volume detected by SRμCT. The expression level of interleukin(IL)-1β and tumor necrosis factor(TNF)-α was significantly decreased after intrathecal injection of the injured CSF-Exos. Especially, a pro-angiogenic protein called cathelin-related antimicrobial peptide (CRAMP) was highly expressed in injured CSF-Exos compared to normal CSF-Exos. Further functional assays showed that cramp protein was required for injured CSF-Exos-induced promotion of angiogenic responses of cultured endothelial cells, as well as angiogenesis and anti-inflammatory and neurological functional recovery in injured spinal cord rat.

Conclusion: Collectively, these results provide the first evidence that injured CSF-Exos could enhance the neurological functional recovery after spinal cord injury. The mechanisms underlying exosomes-induced spinal cord

functional improvement involve promoting angiogenesis and attenuating inflammation via transferring DMBT1 protein to the target cell, thus CSF-Exos may offer a novel potential therapeutic agent for spinal cord injury.

Tracking and imaging of extracellular vesicles to meet the high standards of precision medicine

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The knowledge about the fate of administered exosomes is essential to understand their therapeutic role and is a stepping stone to further refinements such as tailoring routes of their delivery or their engineering. Therefore, the tracking of exosomes is pivotal to pursue exosome-based therapies. There are distinct approaches to tracking of exosomes to address a variety of questions. The studies on systemic distribution and elimination of administered exosomes require the highly sensitive assays, but the *in vitro* readout adds a lot of options. It has been shown that plasmid-based expressed fusion protein consisting of Gaussia luciferase and a truncated lactadherin, gLuc-lactadherin is actively routed to exosomes and can be effectively detected in samples of plasma. We have also recently observed that fusion protein of truncated interleukin 6 (IL6) and a new Nanoluc® Luciferase (Promega), a 100 x brighter enzyme than Gaussia is primarily loaded to exosomes and can also report on the exosome pharmacokinetics. Unfortunately, unfavorable spectral properties of both enzymes eliminate them from utility for *in vivo* studies. The local and targeted deliveries of exosomes require tags to be visualized during procedures. Traditionally, radionuclides are an excellent tool for molecular imaging, and 99mTC-HMPAO and 111In-Oxine, which were previously used for leukocyte labeling, proved also to be useful for exosome imaging *in vivo*. The radioactivity is problematic for further *post-mortem* evaluation, tough and inconvenient for preclinical studies. The nuclear medicine approach also suffers from low spatial and temporal resolution. In this context, the dual modality contrast agent: fluorescently tagged superparamagnetic iron oxide nanoparticles (SPIONs) are a very interesting option. The SPIONs are easily detectable by magnetic resonance microscopy (MRI), with a high spatial and temporal resolution. Although, the inherent negative signal produced on MRI scans precludes utility of this method for long-term *in vivo* tracking of exosomes, due to limited specificity. The fluorescent tag also gives a leeway to use them for *post-mortem* detection of exosomes in cells and tissues. We have also recently found that species-specific miRNA can be used for assessment of inter-species label-free exosome trafficking. Summarizing, there are various options for tracking of exosomes, but still more research is needed to push forward the sensitivity as well as provide data on reliability and potential interference of tags with therapeutic activity of exosomes.

Neuronal death signaling and neuroprotection – advances in cerebral ischemia research

CYLD links pathways of apoptosis and necrosis in neuronal death

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In ischemic brain damage, the underlying neuronal death has been linked to both, mechanisms of apoptosis and regulated necrosis. Further, oxidative stress and mitochondrial damage are established key features of ischemic neuronal death but how these are linked to cellular dysfunction and death at the molecular level is largely unknown. More recently, ferroptosis emerged as a key mechanism of oxidative cell death in many different cell types including neurons. Ferroptosis can be induced by inhibition of the cystine/glutamate antiporter (Xc-) or glutathionperoxidase-4 (GPX4) depletion and is characterized by excessive iron-dependent formation of lipid peroxides.

Using pharmacological compounds and genetic approaches (siRNA, CRISPR/Cas9 knockout) we identified a key role for BH3 interacting-domain death agonist (BID)-mediated mitochondrial damage in oxidative cell death thereby connecting the mechanistic concepts of ferroptosis and mitochondrial pathways of regulated cell death. In oxidative cell death, mitochondrial translocation of BID was triggered downstream of lipid peroxidation, and upstream of dynamin-related protein 1 (DRP1), which together with BID determined loss of mitochondrial integrity and function. Further, we identified cylindromatosis (CYLD) as a major sensor of intracellular ROS accumulation which controlled the formation of the RIP1-RIP3 necrosome and detrimental transactivation of BID and DRP1 to the mitochondria. We exposed mitochondrial damage as the "the point of no return" in paradigms of ferroptosis in neuronal and non-neuronal cells using the mitochondria-targeted antioxidant mitoquinone (MitoQ), which rescued mitochondrial function despite mitochondrial fragmentation and pronounced cytosolic reactive oxygen species (ROS) formation.

Overall, our findings provide novel molecular links between oxidative stress, pathways of regulated necrosis and mitochondrial demise in vitro and in models ischemic brain damage in vivo. According therapeutic approaches targeting mitochondria and upstream mechanisms of regulated necrosis may hold promising treatment strategies for neuroprotection.

Resilience and vulnerability to ferroptosis in hemorrhagic stroke

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Ferroptosis, a non-apoptotic form of prgrammed cell death is dysregulated in cancer, heat stress in plants and neurological disorders, including intracerebral hemorrhage, a stroke subtype. The emergence of ferroptosis as a mechanism for neuronal loss following ICH has provided some clarity about how oxidative stress acts as a signal not a toxin to trigger regulated necrosis. It has also provided opportunity to understand how neurons adapt to stresses (e.g. hemin/Hgb) that drive injury in ICH. These adaptive mechanism are driven by the transcriptional activators TFAP2C and Sp1 acting sequentially to drive a casette of genes involved in protection and repair from ICH induced-neuronal death in vitro and in vivo. Finally, I will discuss an translatable therapeutic strategy that fosters adaptive transcription post ICH, reduces ferroptosis and improves functional recovery.

Key players in neuronal death – past and future.

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The human cerebral cortex is a complex structure with tightly interconnected excitatory and inhibitory neuronal networks that function in an interconnected manner in both physiologic and pathophysiologic actions. In order to study cortical function, neurotoxicity and neuroprotection and to develop clinically relevant therapies it is important that the models used to study disease express this complex network. Technical advances now permit the extension of studies performed in rodent models into human neuronal cultures and also permit the evaluation of the glial contribution to neurotoxicity. We developed a human cortical neuron culture system that expresses both excitatory and inhibitory neuronal networks resembling the composition of the human cortex. We observe neuronal populations representative of the six cortical layers and a network that is functional and homeostatically stable. In human cortical neuron cultures, excitotoxicity or ischemia due to oxygen and glucose deprivation leads to cell death that is dependent on N-methyl-D-aspartate receptors, nitric oxide, and the poly (ADP-ribose) polymerase (PARP)-dependent cell death, a cell death pathway designated parthanatos. Apoptosis inducing factor and macrophage migration inhibitory factor partner to fragment DNA. Inhibition of these cellular events provides neuroprotection. These cultures also undergo preconditioning induced neuroprotection. This culture system provides a new platform for the study of human cortical neurotoxicity and neurodegeneration. Additionally, neuroprotective strategies relevant to the human brain can be identified. Activated microglia can convert astrocytes into a neurotoxic A1 phenotype which are observed in stroke and a variety of neurological diseases. Inhibition of this conversion provides neuroprotection. Future therapies will likely combine both neuroprotection and anti-inflammation approaches.

Non-neuronal mechanisms underlying vascular contributions to cognitive impairment and dementia

Insights from Optical Imaging and Manipulation of Brain Pericytes

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Cerebrovascular resistance tunes the rate of blood flow to the brain and its dysregulation leads to metabolic insufficiency and injury during brain disease. Contractile smooth muscle cells on arteries and arterioles confer vascular resistance. However, >90% of the brain"s microvasculature consists of capillaries covered by capillary pericytes, which have an uncertain role in blood flow control. Using two-photon optogenetics, we show that stimulation of capillary pericytes in the live mouse brain drives their contraction and impedes capillary flow, even in regions distant from the source arteriole. Capillary pericyte contraction is slower and smaller in magnitude than α-smooth muscle actin-rich mural cells of pre-capillary and pial arterioles. Conversely, optical ablation of capillary pericytes resulted in persistent dilation and increased capillary flow. Thus, capillary pericytes are sufficient and necessary to alter capillary tone *in vivo*. Their slower kinetics suggests a role for maintenance of flow resistance throughout the brain"s capillary network.

Targeting astrocyte signaling in VCID

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Astrocyte activation is a complex and functionally elusive process associated with nearly every form of neural injury and neurodegenerative disease. To elucidate the role of astrocyte activation in conditions like traumatic brain injury (TBI) and Alzheimer"s disease (AD), our previous work exploited the functional status of the calcineurin/NFAT pathway, which is often upregulated in activated astrocytes. Together, calcineurin (a protein phosphatase) and NFAT (a transcription factor) exert broad control over gene expression and play a pivotal role in phenotype switching, among other things. Using AAV to deliver an NFAT inhibitory peptide to astrocytes of intact rodent models of TBI and AD, we showed that astrocyte activation is a causative factor for synapse dysfunction and cognitive loss. Here, we discuss our investigations of calcineurin/NFAT signaling associated with astrocyte activation in conjunction with vascular pathology (e.g. microinfarcts) and determine whether selective inhibition of astrocytic calcineurin/NFATs protects against synaptic deficits, as well as vascular dysfunction in a hyperhomocysteinemic mouse model of vascular contributions to cognitive impairment and dementia.

Intramural Periarterial Drainage Pathways and the pathogenesis of neurological diseases

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Accumulation of the β-amyloid (Aβ) protein in cerebral blood vessels is a hallmark of Alzheimer"s disease. Soluble Aβ from the extracellular spaces of the brain is removed along the basement membranes of capillaries and basement membranes surrounding smooth muscle cells of arteries towards the surface of the brain, as intramural periarterial drainage (IPAD). This process depends on the biochemical integrity of the extracellular matrix and the strength of arterial smooth muscle cells. With ageing, possession of Apolipoprotein E4 (APOE4) genotype, hyperlipidemia, maternal high fat, immune complexes, IPAD fails, resulting in the accumulation of proteins in the walls of cerebral arteries as cerebral amyloid angiopathy. Head injury results in changes in the extracellular matrix and accumulation of perivascular tau, possibly as a result of a failure of IPAD. Within 5 minutes of intracisternal injection, convective influx/glymphatic entry of Aβ from the cerebrospinal fluid into the cerebral parenchyma is along the glial-pial basement membranes and enters IPAD by 30 minutes. Clusterin (Apolipoprotein J) appears to be a chaperone for Aβ, facilitating IPAD and efficient innervation of cerebral arteries is key to maintaining optimal IPAD.

Neuroinflammatory and angiogenic factors as biomarkers of VCID

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Vascular cognitive impairment and dementia (VCID) is the second leading cause of dementia and often occurs co-morbidly with Alzheimer"s disease (AD). VCID is also the most common co-morbidity with AD pathology in the late-onset sporadic AD population. Currently, diagnosis for VCID is limited to clinical signs of cognitive impairment along with white matter disease identified on MRI imaging. As novel therapeutics are developed aimed at disease modification, there is an emerging need for biomarkers to accurately identify the patient population that will benefit from the therapies. As part of the MarkVCID Consortium, we have been exploring novel fluid and imaging biomarkers that may be predictive of VCID in a clinical cohort enriched for individuals with high cerebrovascular disease.

In a cohort of 115 individuals with longitudinal CSF and plasma, along with clinical, cognitive, and MRI volumetric white-matter hyperintensities, we examined 29 proteins in the CSF and plasma MSD V-Plex Vascular Injury Panel 2: (SAA, CRP, VCAM-1, ICAM-1. FGF, Flt1, PIGF, Tie-2, VEGF-A, VEGF-C, VEGF-D. IFNg, IL10, IL12p70, IL13, IL1b, IL2, IL4,

IL6, IL8, TNFa, MMP1, MMP3, MMP9, MMP2, MMP10). The data was analyzed using standard machine learning approaches with AdaBoost and Random Forest being applied for feature selection. We identified a collection of 9 features that predicted cognitive impairment in our VCID cohort with an 80% accuracy. These were CSF PIGF, MMP2, SAA, CRP, and plasma MMP9, ICAM-1, VEGF, VCAM-1, VEGF-D. We also have identified a collection of 7 features that provide a 70% predictive value for white matter hyperintensity volume. These were CSF IL8, IL6, VEGFD, PIGF, and plasma TNFa, IL10, MMP10.

In summary, we have identified a series of novel fluid biomarkers that are predictive, in a machine learning model, of severity of VCID.

Targeting the hematoma in intracerebral hemorrhage

Cell type-specific mechanisms of hematoma toxicity in intracerebral hemorrhage

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It is established that adverse outcomes after intracerebral hemorrhage (ICH) result from irreversible damage to neurons resulting from primary and secondary injury. Secondary injury has been attributed to hemoglobin and its oxidized product hemin from lysed red blood cells. However, our advances in understanding neuronal demise after ICH have not translated into effective therapeutic approaches.

There are many possible explanations for the lack of success of current therapeutics at the bedside. One reason may be that they primarily focus on neurons and, more specifically, on neuronal cell bodies. We here hypothesize that the molecular mechanisms underlying cell death and degeneration may be different in different cell types as well as compartments of the cells, such as the axon in comparison to the soma.

We investigated cell death mechanisms in cultured primary neurons, isolated axons, and primary endothelial cells exposed to hemin. We show that different cell death pathways are activated in these cell types or compartments using a systematic screening of chemical inhibitors implicated in known cell death pathways.

This indicates that different therapeutic approaches addressing the numerous types of brain cells are needed to effectively treat patients with ICH and, potentially, other neurological diseases.

Therapeutic targeting of nuclear derived toxic lipids after intracerebral hemorrhage

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Intracerebral hemorrhage (ICH), a stroke subtype, remains a significant cause of mortality and morbidity around the world. Accordingly, there is a need to identify therapeutic agents with known safety in humans such as N-acetyl cysteine (NAC) to expand options for treating ICH. Here, we tried to understand NAC efficacy in models of ICH and to identify the target of its action as a first step toward defining the optimal dose *in vivo* for functional recovery. We used chemical biology, targeted lipidomics, 5-lipoxygenase (ALOX5) knockout mice and viral-gene transfer to gain insight into the pharmacological targets and mechanism of action of NAC. We found that NAC is a promising, protective therapy for ICH, which acted to inhibit toxic arachidonic acid products of nuclear ALOX5 that synergized with exogenously delivered protective prostaglandin E2. Thus, our findings provide novel insight into a target for NAC, beyond the generic characterization as an antioxidant, resulting in neuroprotection and offer a feasible combinatorial strategy to optimize efficacy and safety in dosing of NAC for treatment of neurological disorders involving ferroptosis such as ICH.

Interrogation of human immune responses after intracerebral hemorrhage

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Recent advances in experimental modeling have highlighted immune responses as critical mediators of both injury and recovery after intracerebral hemorrhage. However, little is known about the immune responses within the brains of living human patients. This presentation will discuss human data, including RNA sequencing of leukocyte populations in the peripheral blood and hematoma evacuates, single cell level data in leukocytes, and evidence of pathways identified in animal models in living human patients.

Opening Ceremony

Vascular contributions to cognitive impairment and Alzheimer's disease: innate and adaptive immunity take center stage

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The brain lacks energy reserves and is vitally dependent on a continuous and well-regulated delivery oxygen and glucose through the cerebral blood supply. Structural and functional alterations of cerebral blood vessels have emerged as a key correlate of brain diseases associated with cognitive impairment. Owing to the blood-brain barrier, the brain has traditionally been considered an "immune privileged" organ, nearly impenetrable to immune cells. However, a growing body of evidence indicates that cells of the immune system traffic in and out of the brain and can contribute to a wide variety of brain pathologies, including those associated with impaired cognition and dementia. In this presentation, the mechanistic bases underlying cognitive impairment will be examined focusing on the role of neurovascular dysfunction, as well as innate and adaptive immunity. Emerging evidence indicates that activation of innate immunity, vascular oxidative stress and inflammation are major pathogenic factors in the neurovascular dysfunction induced by arterial hypertension and Alzheimer pathology. On the other hand, the deleterious effects of dietary salt, a key contributing factor to stroke and dementia, involves a gut-initiated adaptive immune response leading to cerebral endothelial dysfunction and cognitive impairment. The recently-appreciated links between innate and adaptive immunity, neurovascular dysfunction, and cognitive impairment represent a fruitful area of research with major diagnostic, preventive, and therapeutic implications for both vascular and neurodegenerative dementias.

Hot news I

Role of NOX5 linked to a NOS-NOX network pharmacology-based combinatory therapy

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Ischemic stroke is the first cause of disability worldwide where no neuroprotective therapy is currently available. Reperfusion post-stroke bears the risk of an acute deleterious calcium-dependent breakdown of the blood-brain barrier. Here we identify the type 5 NADPH oxidase (NOX5), a calcium-activated, reactive oxygen species (ROS)-forming enzyme as a missing mechanistic link in this clinical relevant scenario. *In vitro,* we find upon re-oxygenation or calcium overload brain ROS levels to be increased and in a NOX5-dependent manner. Consistently, *in vivo*, post-ischemic ROS formation, infarct volume and neuro-motor outcomes were worsened in NOX5 knock-in mice. In translation to the clinics, we used a human cell-based blood-barrier barrier (BBB) model of brain ischemia where pharmacological NOX inhibition prevented acute re-oxygenation induced leakage. Thus, our data establish NOX5 as essential to induce calcium-dependent blood-brain barrier breakdown.

However, "one-disease, one-target, one-drug" strategies dramatically failed in the stroke field within the last decades. To overcome this failure, we propose a novel strategy so-called network pharmacology, focused on mechanistic-related synergic targets, which suggests that multi-factorial diseases like brain ischemia should be treated by simultaneous modulation of several targets. Here we extend the single target, NOX known to cause blood-brain barrier breakdown and neurotoxicity, to a network pharmacology approach. We identify the nitric oxide synthase (*Nos1-3*) gene family as the closest network pharmacology target associated with stroke. Importantly, when combining sub-threshold concentrations of a NOS and a NOX inhibitors, cell death was reduced in supra-additive manner suggesting indeed a mechanism-based network pharmacology phenomenon. We then validated this approach in a clinically relevant *in vivo* model of ischemic stroke. Again, sub-threshold doses of NOX and NOS inhibitors synergistically reduced infarct size, stabilized the BBB and preserved neuro-motor function. Finally, extending these findings to a human BBB model our network pharmacology therapeutic approach was fully preserved.

NOS1, NOX4 and NOX5 are therefore synergistic and mechanistic-related targets in ischemic stroke. This NOX-NOS network pharmacology approach provide a rational strategy to reduce the risk of failure in drug development increasing both efficacy and safety for unmet medical need indications. We therefore suggest to clinically validate post-stroke recanalisation in the presence of a NOS-NOX inhibitor.

Inflammatory events and acute endothelial responses to focal ischemia

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Question: Circulating complement factors are activated by tissue damage and contribute to acute brain injury. Following focal ischemia, the deposition of mannose-binding lectin (MBL), one of the initiators of the lectin complement pathway, on the cerebral endothelium is regarded as a major pathogenic event leading to brain injury. The study addresses the molecular mechanisms through which MBL influences outcome after ischemia.

Methods: Wild type (WT) or MBL-/- mice underwent 30" middle cerebral artery occlusion (tMCAo) or sham surgery to assess: 1) hemodynamics by in vivo two-photon microscopy, 2) expression of C3b, thrombomodulin, ICAM and CD206 by western blot and immunohistochemistry, 3) platelet and blood cell cytokine levels by ELISA. To mimic ischemic injury in vitro, cultured human brain endothelial cells were subjected to oxygen-glucose deprivation (OGD) and cell death and CXCL1 release were measured. In a further set of experiments MBL deposition on ischemic vessels was determined by immunofluorescence in tMCAo mice treated with IL-1 receptor antagonist.

Results: We show that after tMCAo, MBL-/- mice had better flow recovery and less extravasation than WT mice. They had also reduced plasma C3b, increased levels of thrombomodulin on brain vessels and thrombomodulin lectin-like domain in plasma. MBL-/- mice also presented reduced expression of ICAM-1 and increased number of CD206+ cells, overall indicating an attenuated proinflammatory vascular phenotype.

We also show that platelets directly bind MBL and that platelets from MBL-/- mice have reduced inflammatory phenotype as indicated by reduced interleukin-1α (IL-1α) content, as early as six hours after ischemia. Endothelial cells subjected to OGD and exposed to platelets from MBL-/- mice present less cell death and lower CXCL1 release (downstream to IL1-α) than those exposed to WT platelets. In turn, MBL deposition on ischemic vessels significantly decreases after ischemia in mice treated with IL-1 receptor antagonist compared to controls, indicating a reciprocal interplay between MBL and IL-1α facilitating endothelial damage.

Conclusion: Thus MBL which drives detrimental vascular responses following cerebral ischemia, acts as an early trigger of platelet IL-1α release which in turn favours MBL deposition on ischemic vessels promoting an endothelial pro-inflammatory phenotype. The study shows the existence of novel targets in brain ischemic injury

Therapeutic angiogenesis for neurorepair

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Microvasculature is the key element that maintains neural activity in full. Major causes of dementia in elderly, including impaired amyloid/tau protein clearance, increased blood brain barrier permeability and cerebral ischemia, are closely linked with impaired cerebral microvasculature function.
We had been shown that therapeutic angiogenesis with hematopoietic stem cells improves brain function both in experimental stroke model and clinical trial for stroke patients. Recently, we have identified the mechanism how hematopoietic stem cell regenerate injured microvasculature function. Based on these observations, we have

started project that focus on improvement of brain function through therapeutic angiogenesis in patients with dementia.

Adaptive immune cell activation in acute pediatric traumatic brain injury

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Question: Traumatic brain injury (TBI) arises in two phases: the first is caused by mechanical injury to the brain resulting in tissue shearing, bleeding, and cerebral ischemia, and a secondary phase mediated by excitotoxicity, edema, delayed axonal injury, and neuroinflammation. In fact, chronic inflammation following TBI predisposes individuals to other neurological diseases that are particularly relevant for pediatric TBI (pTBI). Clinical studies investigating neuroinflammation are limited and rarely focus on adaptive immune responses in pTBI patients.

Methods: Pediatric TBI patients (1-18 yrs.) and controls (0-18 yrs., no CNS injury, n=10) were recruited according to approved IRB protocol. Blood and cerebral spinal fluid were collected at days 1, 3 (both n=14) and day 5 (n=13) following TBI and placement of an external ventricular drain. Peripheral blood immune cells were enumerated using fluorescent antibodies and flow cytometry. Statistical analysis used Kruskal-Wallis non-parametric one-way ANOVA for comparison between pTBI and controls, while Friedman non-parametric one-way ANOVA was used for between day comparisons. Neuronal autoreactivity was evaluated by labeling peripheral blood mononuclear cells with carboxyfluorescein succinimidyl ester (CFSE) and culturing with neuronal and control antigens. Response to antigen was determined by dilution of CFSE (proliferation) and expression of CD25 (activation).

Results: pTBI patients exhibited a higher quantity of leukocytes ($CD45^+$) relative to controls (p=0.01) on day 1 post-TBI, indicating an immediate and robust immune modulation due to CNS injury, that decreased at days 3 and 5. Major subsets of adaptive immune cells (CD3, CD19, CD4, and CD8) did not exhibit altered cellularity relative to controls but activated adaptive T cells ($CD4^+CD161^+$and $CD8^+CD161^+$) were substantially higher at day 1 (p=0.01 and p=0.03, respectively). Autoreactivity analysis of pTBI patients (n=3) revealed a CD4 T cell response to various CNS-derived antigens (e.g. MAP2, PLP, β-amyloid) which is absent in controls (n=3). Interestingly, the CD8 T-cell analysis revealed CNS-specific autoimmune responses absent in pTBI patients which were present in controls.

Conclusions: The observable differences within the $CD45^+$, $CD4^+CD161^+$and $CD8^+CD161^+$cell populations showed alterations in cellularity that coincided with CNS injury. This may suggest alterations in cellular tracking as CNS injury evolves. Detection of CNS-specific CD4 T-cells and loss of CNS-specific CD8 T-cells suggest specific activation of adaptive immune cells which may have reciprocal roles in brain injury and/or repair. Based on these initial observations, we hypothesize that the adaptive immune system is activated upon TBI and can potentially target CNS resident cells in young patients. Thus, these data warrant further investigation into the role of the adaptive immune system after brain injury and its potential role in immunotherapeutic targeting.

Hot news II

Secretome profiles of cytokines and chemokines during oxygen deprivation indicate that rat astrocytes but not brain pericytes contribute to inflammatory response

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Introduction: Oxygen deprivation (OD) in the brain triggers inflammation. Though microglia / resident macrophages play a major role, the precise mechanisms of the inflammatory responses after OD *in vivo* are difficult to elucidate because of the complex nature of multiple series of interactions between cells and signalling molecules. In order to elucidate whether signalling from astrocytes and brain pericytes contributes to inflammatory response, this study explored temporal patterns of secretion of 30 cytokines and chemokines from these cells in primary culture that were exposed to OD and then associated changes in secretion to potential molecular networks.

Methods: Primary cultures of brain astrocytes and pericytes from Sprague Dawley rates were produced as explained earlier and incubated for periods of 2-24h in the absence of oxygen or under normal partial pressure of oxygen (controls). Simultaneous detection of 29 cytokines and chemokines in the samples was performed using a rat cytokine array panel, while the temporal pattern of angiopoietin-1 (Ang-1) secretion was determined separately using ELISA. Wilcoxon-Mann-Whitney test was used to compare normoxic and anoxic samples and the Hodge-Lehman estimator with exact 95% confidence intervals was computed to assess the size of differences in cytokine secretion. The obtained data were imported into the Core Analysis tool of Ingenuity Pathways Analysis software in order to relate changes in secretion of cytokines and chemokines from astrocytes during OD to potential molecular signal networks.

Results: With the exception of Ang-1, which concentrations significantly decreased, concentrations of all other 29 cytokines/chemokines in samples collected from astrocytes after OD were either the same, or significantly higher, than in control groups. No clear pattern of changes could be established for groups of cytokines with similar effects (i.e. pro- or anti-inflammatory cytokines). Contrary to that, concentrations of all cytokines/chemokines in samples collected from pericytes after OD were either the same, or marginally lower, than in control groups. Analysis by Ingenuity Pathways Analysis software idicated that the pattern of changes in cytokine secretion from astrocytes during oxygen deprivation was associated with the HIF-1α-mediated response and enhancement of inflammation through IL- 1β and cathepsin S pathways.

Conclusions: These *in vitro* findings suggests that astrocytes but not brain pericytes contribute in triggering inflammation during OD in the brain.

Advanced theranostic nanocarrier-mediated delivery of NGF in a combination therapy stimulates recovery after stroke

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Question: The majority of people who survive the acute phase of stroke remain permanently disabled. So far no treatment to improve function exist, nerve growth factor (NGF) has emerged as excellent candidate to boost recovery processes. However, the approach to effective deliver NGF has not been possible since it does not pass the blood brain barrier. The blood brain barrier is a significant obstacle and the limiting factor in delivery of growth factors into the brain. To overcome this hurdle, the overall aim of this study is to provide safe and efficient targeted brain delivery of growth factors using a smart nanotechnology-based solution that will stimulate brain recovery

processes. We have previously shown that mitogen activated protein kinase (MEK)1/2 inhibition promotes recovery after stroke. The aim of this study was to use an advanced theranostic nanocarrier-mediated delivery of NGF to evaluate if combination therapy (U0126 in acute phase together with NGF in subacute phase) have more beneficial outcome after stroke compared to single treatment by U0126.

Method*:* Advanced theranostic nanocarrier-mediated delivery of NGF was developed. Transient middle cerebral artery occlusion was induced in male rats for two hours followed by reperfusion. The specific MEK1/2 inhibitor U0126 was administered i.p at 6 and 24 hours and the NGF was given i.v at day 3 post-reperfusion Neurological functions were evaluated by 28-point tests and 9.4 T magnetic resonance imaging was used to monitor morphological infarct changes.

Results*:* In the present study, biodegradable nanocarriers comprising human serum albumin and NGF have successfully been used to deliver a growth factor into the brain. The combination therapy with NGF and U0126 significantly improved neurological function and reduced infarct compared to vehicle and U0126 treatment alone, 4 weeks after treatment.

Conclusion*:* NGF delivered by advanced theranostic nanocarrier promotes recovery processes after stroke and most importantly our delivery approach has demonstrated to be successful. This will be the first step that will lead to novel treatment strategies for neurological disorders.

Cell Therapy and Coagulation

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As clinical trials using cell therapies grow in prevalence and scale, additional concerns about the safety of cell therapies have been reported. Several recent reports have demonstrated the potential of cell therapy, and particularly mesenchymal stem cells, to affect coagulation both using *in vitro* and *in vivo*. We recently described the relationship between tissue factor (cd142) expression across clinical cell therapies from a number of donors and tissues and an increase in coagulation. In recent work, we have evaluated the ability of clinical anti-coagulants to manage risk of thrombosis when using cell therapies for at risk patients.

Postacute delivery of GABAA α5 antagonist promotes postischemic neurological recovery and periinfarct brain remodeling

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Background and purpose: Poststroke, neuronal excitability is tonically reduced in periinfarct tissue via inhibitory influences of extrasynaptic GABAA receptors. We hypothesized that GABAA α5 blockade by the competitive antagonist S44819 enhances postischemic neurological recovery, brain remodeling and neuroplasticity.

Methods: In an explorative study followed by a confirmation study, male C57Bl6/j mice were exposed to transient intraluminal middle cerebral artery occlusion. Starting 72 hours poststroke, vehicle or S44819 (3 or 10 mg/kg, twice daily) were delivered per os over 28 days. Neurological recovery, perilesional tissue remodeling and contralesional pyramidal tract plasticity were evaluated over 42 days, i.e., up to 14 days after completion of S44819 delivery.

Results: S44819, delivered at 10 but not 3 mg/kg, persistently improved motor-coordination and spatial memory in both studies. Striatal atrophy was reduced by 10 mg/kg S44819 at 42 days post-treatment onset, and neuronal long-term survival in the periinfarct striatum was increased. Delayed neuroprotection was associated with reduced periinfarct astrogliosis, increased periinfarct brain capillary density and increased neural precursor cell proliferation and differentiation in proximity to the ipsilesional subventricular zone. Contralesional pyramidal tract plasticity, evaluated by anterograde tract tracing at the level of the red nucleus, was not influenced by S44819. Concentrations of neurotrophic (BDNF, GDNF) and angiogenic (VEGF, FGF) growth factors were elevated by 10 mg/kg S44819 in periinfarct, but not contralesional brain tissue.

Conclusion: Our data demonstrate that S44819 enhances neurological recovery and periinfarct brain remodeling in the postacute stroke phase. Based on these data, a randomized controlled multicenter phase IIb trial, RESTORE Brain (https://clinicaltrials.gov/ct2/show/NCT02877615), is currently performed in fourteen countries in five continents, the data of which are expected in 2019.

Chronic cerebral hypoperfusion accelerates Alzheimer's disease pathology with cerebrovascular remodeling in a novel mouse model

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Aging societies show an increasingly strong relationship between Alzheimer's disease (AD) and chronic cerebral hypoperfusion (HP). In the present study, we created a new mouse model for AD plus HP, and investigated its clinical and pathological characteristics. Alzheimer's disease transgenic mice (APP23) were subjected to bilateral common carotid arteries stenosis with ameroid constrictors for slowly progressive cerebral HP. In contrast to simple APP23 mice, cerebral HP exacerbated motor and cognitive dysfunctions with white matter lesions and meningo-parenchymal amyloid-β (Aβ) burdens. Strong cerebrovascular inflammation and severe amyloid angiopathy with cerebrovascular remodeling were also observed in APP23 + HP mouse brains. An acetylcholine esterase inhibitor galantamine improved such clinical dysfunctions, retrieved above neuro- pathological characteristics, and enhanced nicotinic acetylcholine receptor (nAChR)-binding activity. The present study demonstrates that chronic cerebral HP enhanced cognitive/motor dysfunctions with parenchymal/cerebrovascular Aβ accumulation and cerebrovascular remodeling. These neuropathological abnormalities were greatly ameliorated by galantamine treatment associated with nAChR-mediated neuroprotection by allosterically potentiating ligand action.

Correlating multimodal in vivo imaging of neurorepair elements after ischemic brain lesion in the mouse

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In vivo imaging of the small experimental animals (i.e. mice and rats) allows monitoring and evaluation of the pathological processes and corresponding interventions in the living animals through extended time, in a similar way the diseases are followed in the human patient. We used this approach to assess the repair after ischemic brain lesion in the mouse, as a model for the ischemic stroke in humans. The imaging modalities used included small animal magnetic resonance imaging (MRI), preclinical optical imaging with emphasis on bioluminescence imaging (BLI), and synchrotron radiation and laboratory-based X-ray micro computed tomography (SRμCT and μCT). We assumed that through combining the modalities the previously unrecognized differences between tested (reduced neruoinflammation) and control group can be revealed, subsequently giving insight in consequences of neuroinflammation on brain repair.
Medial cerebral artery occlusion (MCAO) for 60 minutes followed by reperfusion was performed on 3 months old mice. The ischemic lesion was evaluated by magnetic resonance imaging (MRI, Bruker 7T Biospec 70/20 USR) and bioluminescence imaging (BLI, Perkin Elmer IVIS Spectrum). Gap43 and Casp3/7 were used as molecular markers. As

Toll-like receptors located on microglia are key initiators of inflammatory response, Tlr2 loss of function mice (i.e. tested group) was used as a model for reduced neuroinflammation after ischemic lesion. The imaging data were complemented by functional tests and Western blot protein analysis of the brain samples.
The multimodal imaging advantage was to correlate the photon flux recorded by BLI to the lesion size obtained by MRI. This allowed normalizing the bioluminescence signal among different animals, which resulted in statistically significant differences between Tlr2-deficient and control group. Gap43 (marker of axonal outgrowth) and Casp3/7 (apoptosis and neuronal stress) bioluminescence was used to evaluate elements of repair after ischemia. In Tlr2-deficient mice, Gap43 bioluminescence was higher until 28 days after ischemia, and Casp3/7 increased 14 days onward as compared to the wild type animals. Synaptic markers DLG4 and synaptophisin were higher in Tlr2-deficent mice.
Reducing the neuroinflammation in Tlr2 loss of function mice increased Gap43 and Casp3/7 activities, accompanied by an increase in synaptogenic markers. Bioluminescence imaging combined with magnetic resonance served in this multimodal approach to assess brain repair after ischemic lesion in the mouse.
Acknowledgments: This work was funded by FP7 GlowBrain, ESF Young Brain, HamagBicro POC6-1-153 and CSF grant RepairStroke. Multimodal imaging was done at Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb School of Medicine.

Neuroprotection and neurorepair

The role of short-chain fatty acids in post- stroke regeneration.

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Questions: The gut harbours a readily adapting bacterial population known as the microbiome. The gut microbiome has been shown to alter the outcome of many brain diseases including stroke. Previous reports have shown that microbial short-chain fatty acids (SCFA) are capable of polarising T cells towards an anti-inflammatory phenotype (Treg), which have been found to be neuroprotective in stroke. Moreover, SCFA have been associated with microglial activation states. This study investigates the ability of SCFA to modulate stroke outcome and the involved mechanisms.

Methods: We analysed SCFA concentrations in various organs by NMR. Therapeutic SCFA supplementation was achieved by administration of acetate, butyrate and propionate in the drinking water. Neuronal spine density was investigated by Golgi-Cox staining. Microglial activation was determined by morphology analysis using an automated 3D shape analysis. Both neuronal and microglial changes were further characterized using qPCR for key markers involved in neuronal plasticity and microglial activation. The impact of SCFA treatment on the post-stroke transcriptional profile was determined by mRNA sequencing and RT-PCR for candidate validation. To investigate the immune cell polarization induced by SCFA, we performed flow cytometry of the intestinal immune system and brain. We utilized three different focal stroke models in order to determine changes in infarct volume and post-stroke behaviour using an automated assay of distal forelimb function. Finally, by performing calcium imaging of Thy1-GCaMP6 transgenic mice, we were able to observe functional neuronal activity and detect changes in connectivity up to day 70 after stroke.

Results: We found that germ-free or antibiotic treated mice had significantly reduced SCFA levels. We observed that in naïve SPF mice, the spine density of neurons was higher and the microglia morphology was more branched in SCFA- compared to control treated groups. In contrast, after stroke we saw no changes in cortical network function, infarct volumes or behavioural deficits. In line with this, we saw no change in spine density within the ipsilateral cortex 14d after photothrombosis. However, changes in the contralateral hemisphere were still evident. The microglia in SCFA-treated mice were more branched in the ipsilateral hemisphere compared to controls, indicating

a reduced activation state. In addition, mRNA sequencing demonstrated that mice treated with SCFA had an increased expression of the lysosomal protein LAPTM5, which is highly expressed in microglia. Finally, flow cytometry of post-stroke brains revealed a general reduction in the cerebral leukocyte count of SCFA-treated animals.

Conclusions: This study has shown that SCFA treatment altered the neuronal spine density, immune cell numbers, microglia transcriptional profile and morphology in the brain; however, these changes did not lead to changes in infarct volume or functional connectivity after stroke.

Inhibition of nuclear factor-κB activation is the key event of anti-inflammatory and protective effects of activated protein C at mast cells and neurons.

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A key transcription factor - nuclear factor-κB (NF-κB) involved in regulation of genes encoding pro-inflammatory and pro-apoptotic factors, can be activated by a variety of stimuli, including inflammatory cytokines. Brain injury is associated with neuroinflammation, neurodegeneration, and also blood coagulation with thrombin formation and generation of activated protein C (APC).

In the present study we investigated the ability of APC to modulate the production of IL-1b and IL-6 by mast cells (MC), MC survival and NF-κBp65 translocation into MC nucleus in acute inflammation in rats and at glutamate(Glu)-induced toxicity in cultural neurons.

Acute inflammation was induced by intraperitoneal injection of thioglycolate broth (2 ml 40% w/v) in rats. Crude MC were isolated from the peritoneal cavity of anesthetized rats and purified in Ficoll density gradient. IL-1b, 6 in rat were analyzed using Rat IL-1β and IL-6 ELISA Kit (PeproTech, USA). We used immunoassay and immunostaining with confocal microscopy and antiNF-κBp65 antibody for analyze the translocation of NF-κBp65 to nucleus. MTT-test was used to evaluate MC viability at inflammation and neuron survival at Glu, thrombin-toxicities.

Thioglycolate injection induced inflammatory response verified by IL-1β and IL-6 measurements. MC IL-6 production was 1.3, 1.8 and 1.9 fold decreased by single intraperitoneal injection of 5nM APC at 0.5, 1.5 and 4 h of inflammation, respectively. IL-6 level in peritoneal fluid was 1.5 and 1.7 fold decreased in APC-treated rats at 1.5 and 4 h of inflammation. The enhanced production of IL-1β in MC and peritoneal cavity under action of APC was also decreased. APC protected MC from death at 30 min of acute inflammation, increasing the MC survival on 36% (to 89,6 ± 7,6%) compared to the control (53,6 ± 8,7%). The development of inflammation in rats led to activation of NF-κBp65 and its translocation into the nucleus. The maximum of nucleus NF-κBp65 was observed in 1.5 h after induction of inflammation. APC blocks the translocation of NF-κBp65 into the nucleus of MC. The maximal effect of APC was revealed in 1.5 h after induction of inflammation.

We show that APC at concentrations as low as 1–2 nM inhibits translocation of NF-κB p65 into the nucleus of cultured rat hippocampal neurons, induced by 100 M Glu or 50 nM thrombin. The blocking effect of APC on NF-κB p65 translocation was observed at 1 and 4 h after treatment of neurons with Glu, when the NF-κBp 65 level in the nucleus was significantly above the basal level.

The binding of APC to EPCR/PAR-1 is required to control NF-κB activation at Glu-toxicity and acute inflammation in rats.

Our present data indicate that APC provides not only anti-apoptotic protection (in case of Glu-toxicity), but also anti-neuroinflammatory activity (in case of toxicity caused by thrombin and at acute inflammation) via decrease the nuclear level of NF-B p65 in cells.

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The microRNA miR-21 protects the brain from focal ischemic stroke as described by the Stroke Academic Industry Therapeutic Roundtable (STAIR) recommendations by inhibiting pro-inflammatory and pro-apoptotic pathways

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Question: Does miR-21 merit consideration as a potential therapy for stroke?

Methods: The STAIR criteria maximize the impact of stroke research by improving the models for patient and treatment. Accordingly, preclinical investigations must demonstrate neuroprotective potential in aged animals, both sexes, and animals with comorbidities. Notably, the study must consider clinical, functional, and long-term outcomes. Further, treatment must be considered, including dosage, route of administration, and therapeutic window. Using these criteria, we investigated if the miRNA miR-21 is a potential candidate for stroke therapy.

To address our question, we administered miR-21 mimic or negative control miRNA mimic in PEGylated liposomes to groups of C57BL/6J mice subjected to 1 hour middle cerebral artery occlusion (MCAO). Long-term survivorship was analyzed using the Mantel-Cox survival curve comparison. Behavioral outcomes were analyzed using 2-way RM ANOVA. All other analyses used the Mann-Whitney unpaired t-test. In all cases, $p<0.05$ was significant.

To assess neuroprotective capacity, young (13-16 weeks) and aged (15-18 months) mice of both sexes were intracranially injected with mimic at 2 hours prior to MCAO. Clinical outcomes (weight loss and neurological score) were assessed out to 48 hours of reperfusion, when infarct volumes were analyzed. To assess functional outcomes, young males were pre-treated with mimic, then subjected to sensorimotor tests (rotarod and corner test) out to one week of reperfusion, and cognitive rests (novel object recognition and fear conditioning) out to four weeks of reperfusion. To evaluate post-stroke treatment, young males were administered mimic via 1) intracranial route at 2 hours post-MCAO, or 2) intravenous route at 30 minutes post-MCAO. To assess mechanisms behind miR-21 neuroprotection, we used tissues from young males at 48 hours of reperfusion. Fresh penumbra was used for qRT-PCR, and fixed sections were used for immunohistochemical detection of activated microglia and neutrophils.

Results: Pre-stroke treatment with miR-21 decreased stroke lesion volume in young and aged mice of both sexes, but only improved clinical outcomes in young mice. Further, pre-stroke treatment with miR-21 improved sensorimotor, but not cognitive recovery. A greater proportion of miR-21 treated mice survived out to 28 days post-stroke, however this trend was not statistically significant. Post-stroke miR-21 treatment improved clinical outcomes, but only intravenously post-treated mice showed decreased lesion volume. Treatment with miR-21 downregulated 27 previously validated miR-21 target genes that participate in pro-inflammatory and pro-apoptotic functional pathways. We further found that miR-21 treatment downregulated 48 pro-inflammatory cytokines, and decreased microglial activation and neutrophil infiltration.

Conclusions: Overall, the results implicate miR-21 as a promising candidate for further study as a stroke therapy.

Selective Ccr2 gene deletion in myeloid cells impairs spontaneous functional recovery following stroke in mice

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Questions: Immune cells invade the brain after severe ischemic stroke but the role of the specific subsets of infiltrating leukocytes is not well defined. Classically, macrophages were regarded as proinflammatory and detrimental for the ischemic tissue, but novel evidence is challenging this view. Infiltrating macrophages derive, at least in part, from monocytes that are typically marked by low/high Ly6C expression. The chemokine receptor CCR2 is critical for monocyte infiltration and CCR2-deficient mice show smaller lesions and better functional outcome. However, several studies reported that blocking CCR2 exacerbates the ischemic lesion and functional outcome. The limitation of interacting globally with CCR2 is that, in addition to monocytes, other cells, such as T cells, express CCR2. The objective of this study was to investigate the effect of monocyte infiltration into the ischemic tissue by selective deletion of CCR2 in this cell population.

Methods: We generated mice lacking CCR2 in myeloid cells using LysM-cre+ mice and Ccr2flox/flox mice (kindly donated by Dr. Manolis Pasparakis, Cologne, Germany). Given that the expression of CCR2 is low or absent in mouse neutrophils, the major cell populations affected by this genetic deletion are the monocytes. Adult mice CCR2flox/flox cre- (WT) and CCR2flox/flox-cre+ (KO) of both sexes received coagulation of the distal middle cerebral artery (MCAo) mice. We studied the evolution of the brain lesion by longitudinal T2*w* MRI and at the same time points up to 15 days we conducted behavioral tests to assess the neurological function with the rotarod test, grip test, pole test, and the adhesion-removal tape test. Immune cell populations in the brain were studied by flow cytometry at days 1, 4, and 15. We also studied brain gene expression by qRT-PCR.

Results: Mice with CCR2-deficient monocytes showed less infiltration of proinflammatory Ly6Chi monocytes, and to a lower extent, Ly6Clo monocytes after stroke. Neutrophil infiltration was not affected by the gene deletion. Infarct volume was similar in both genotypes. However, mice with CCR2-deficient monocytes showed a lower expression of pro-inflammatory genes, such as IL-1b, TNFα, COX2, MMP3, at 24h. The neurological function assessed with rotarod test and the pole test progressively and spontaneously improved in WT mice from 1 to 15 days after stroke. However, the effect was not observed in mice with CCR2-deficient monocytes, which at 15 days remained as affected as at day 1.

Conclusions: The infiltration of CCR2+ monocytes induces a pro-inflammatory profile after stroke that seems to be necessary to support spontaneous recovery of the neurological function. The study suggests that monocytes mediate some features of the inflammatory response to acute stroke that are required for secondary resolution and repair.

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Long-term organotypic culture of the adult human retina: A new reliable method for studying neuroprotection and neurorepair

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Question: Corneal transplantation replaces diseased or damaged tissue with healthy tissue from an organ donor. While the surgery to harvest transplantable corneas from recently deceased donors removes the whole eye, the rest of the tissue is typically disposed of. We developed a culture technique which maintains retinas from those eyes in excellent condition for more than three months, enabling research on human retinal tissue that was previously impossible.

Methods: Human eyes were collected through scheduled multi-organ donations. Following dissection of the eye ball, organotypic retina cultures were prepared within 1-2 hours after the circulation arrest in the donor. The retinal pieces with or without attached retinal pigment epithelium (RPE) and choroid were cultured for up to 100 days in a specific, serum-free, chemically defined medium which has been optimized for the human retina.

Results: Both isolated neural retina and retina-RPE-choroid co-cultures were astonishingly well preserved morphologically with low inter-sample variability. Every major cell type survived and all retinal layers were maintained even after twelve weeks. The quality and the density of outer segments were superior in retina-RPE-choroid co-cultures. A mean density of 4500-5000 cones/mm^2 were measured even in long-term cultures. Subpopulations of bipolar, horizontal and amacrine cells showed close to normal morphology. Only the number of surviving ganglion cells showed a significant decrease in long-term cultures, but ganglion cells were still present even after 84 days. Synaptic structures showed close to normal morphology on samples stained against synapthophysin, while prominent telodendria on cone pedicles indicated intact gap junctions. While retinal neurons did not undergo severe apoptosis, a gliotic remodelling occured in culture. As a sign of retinal edema, the retinal thickness was increased variably. Müller cells became hypertrophic and showed an increased expression of vimentin and GFAP, while the level of glutamin synthetase decreased significantly. Reactive astrocytes with swollen cytoplasm were found in the inner retina. In non-cultured controls the microglial cells were restricted to the inner retina, while in cultures a fraction of the activated microglia invaded the outer retina.

Conclusions: The adult human retina can be maintained in an appropriate culture system for at least three months. By long-term culturing, both acute and chronic effects of pharmacological compounds could be tested directly on human tissue in a cost- and time-effective manner. Further, the long culture time allows the administration of viral vectors and opens new strategies for developing and testing gene therapeutic approaches and can help to reduce the use of animals both in academic and industrial research.

Post-stroke MANF administration facilitates functional recovery and brain repair

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Stroke is the most common cause of adult disability in developed countries, largely because spontaneous recovery is incomplete. No pharmacological therapy exists to hasten the recovery. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an endoplasmic reticulum (ER) resident protein with neuroprotective actions. Our research questions have been i) How MANF mediates its neuroprotective effects? ii) What is the role of MANF in neuronal stem cells and how it contributes to the development of cerebral cortex? iii) What are the effects of post-stroke MANF administration on functional recovery and cellular reparative mechanisms? We set out to study whether post-stroke MANF administration would enhance brain tissue repair and affect behavioural recovery of rats after cerebral ischemic injury. We have used conditional MANF knock-out mice, in vitro primary cultures, rat distal middle cerebral artery occlusion model of ischemia-reperfusion injury, and administered MANF either as a recombinant protein or via the adeno-associated virus (AAV) vector. We also used peri-infarct targeting method we have recently developed for AAVs post-stroke. We found that when MANF is delivered to the peri-infarct region 2 or 3 days after stroke, it promotes functional recovery of the animals without affecting the lesion volume. Furthermore, MANF treatment enhanced transiently endogenous brain repair processes such as increased the number of phagocytic microglia/macrophages in the subcortical peri-infarct regions as well as increased number of migrating neuroprogenitor cells. The analysis of MANF knockout mice revealed the neuroprotective effects of endogenous MANF against ischemic injury and that MANF is essential for neurite extensions and migration of developing neurons. The beneficial effect of MANF treatment on the reversal of stroke-induced behavioural deficits implies that we should continue developing MANF-based therapies to repair the brain after stroke.

The presentation is based on Airavaara et al., J Comp Neurol 2009; Airavaara et al., Exp Neurol 2010; Mätlik et al., J Neuroci Methods 2014, Mätlik et al, Cell Death & Disease 2015; Tseng et al., eNeuro 2017; Tseng et al., Molecular Therapy 2018; Mätlik et al., Science Advances 2018

Combination treatment with U0126 and t-PA reduces adverse effect of delayed t-PA treatment after stroke

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Question*:* The FDA-approved drug for acute ischemic stroke; tissue plasminogen activator (t-PA) has many limitations, recanalization efficacy is far from optimal and it can only be administered within 4.5 h after stroke onset in order to reduce risk of hemorrhagic transformation. Elevated metalloproteinase (MMP) plasma levels have been reported to correlate with the frequency of hemorrhagic transformation following stroke. Inhibitors of the Mitogen–activated protein kinase kinase extracellular signal–regulated kinase kinase (MEK) 1/2 pathways reduce the MMPs following experimental stroke. The aim of this study was to investigate if the combination therapy of a MEK/1/2 inhibitor and t-PA can prevent the detrimental effects of t-PA treatment in stroke.

Methods*:* Thromboembolic stroke was induced by local injection of thrombin directly into the right MCA of C57 black/6J mice. The specific MEK1/2 inhibitor U0126 was administrated 3. 5 h post stroke-onset and t-PA (Alteplase®) was administrated at 4 h post stroke-onset. After 24 h, all animals were euthanized and MMP-9 and phosphorylated extracellular signal regulated kinase (ERK) 1/2 protein levels were investigated by Western Blot and immunofluorescence. Presence of hemorrhage was verified by histology and MRI. The infarct volume was measured by MRI.

Results*:* Delayed administration of t-PA had deleterious effect versus control. Increased infarct volume, presence of hemorrhage and enhanced MMP-9 protein levels was observed in the stroke group treated with t-PA alone compared to control animals. Combination treatment with U0126 and t-PA prevented hemorrhagic transformation and significantly decreased infarct volume, and MMP-9 protein levels compared to control group. Furthermore, p-ERK1/2 protein expression was blocked after combination treatment compared to control animals.

Conclusion*:* Blocking the MMP-9 by using a MEK1/2 inhibitor is a promising adjuvant strategy to alleviate the detrimental side effects of delayed t-PA treatment.

Dementia and cognitive impairment

Benefits of voluntary aerobic physical exercise on cognition and white matter pathology in a mouse model of vascular cognitive impairment and dementia

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Questions: White matter (WM) pathology is a clinically predictive feature of vascular cognitive impairment and dementia (VCID). Here, we investigated WM changes in a VCID mouse model and determined whether physical exercise (PE) could be protective based on evidence pointing to its ability to delay dementia onset. Transgenic mice overexpressing transforming growth factor-β1 (TGF mice) with an underlying cerebrovascular pathology were fed a high cholesterol diet (HCD) to trigger cognitive deficits. We investigated WM pathology and mechanisms underlying the benefits of PE.

Methods: Six groups of equally distributed male and female mice (*n* = 20-24 per group, 3-4 months old) were used and all *in vivo* experiments were performed blind to the identity of the mice. Groups consisted of wild-type (WT)

and TGF mice fed standard lab chow (control groups), WT and TGF mice fed a 2% HCD, WT and TGF mice fed a HCD and concurrently given 3h of access to running wheels. After 3 months of treatments, behavioural tests were performed: Morris water maze, novel objection recognition, and alternation Y-maze. Fluorescent-activated cell sorting experiments were conducted to investigate peripheral immune cell infiltration in WM areas. Laser Doppler flowmetry was performed to measure cerebral blood flow (CBF) responses evoked by whisker stimulation, whereas baseline CBF was measured in WM areas with [14C]-iodoantipyrine autoradiography in a small number of mice. WM functionality was measured in micro-dissected corpus callosum using *in vitro* electrophysiology to record compound action potential amplitude and conduction velocity. In order to study anatomical changes, a subset of mice was intracardially perfused for immunohistochemistry analysis. Two-way ANOVA followed by Newman-Keuls multiple comparison test were performed.

Results: The HCD had a statistically significant effect in both WT and TGF mice that was prevented by PE on the novel object recognition task, the same was observed but only in TGF mice for the Y-maze. Both baseline WM CBF and sensory-evoked CBF increases were reduced in VCID mice, deficits that were countered by PE. VCID mice displayed focal WM functional deficits characterized by lower compound action potential amplitude, which were not found in PE groups. In addition, markers of WM pathology in VCID mice such as increased number of collapsing capillaries, microglial activation associated with WM damage, and reduced number of oligodendrocytes, were all prevented by PE.

Conclusions: Our findings suggest that targeting WM pathology in VCID may be key to improving cognitive symptoms and that regular aerobic PE is an effective preventative treatment. It is possible that reduced CBF in conjunction with increased WM inflammation and reduced number of oligodendrocytes resulted in memory impairments. Increased CBF and oligodendrocyte maturation, and reduced WM-associated inflammation by PE may have helped overcome cognitive deficits induced by HCD.

Angiotensin II type 2 receptors failed to rescue cognitive decline but had selective cerebrovascular benefits in a mouse model of Alzheimer's disease

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Questions: The risk of developing Alzheimer's disease (AD) increases with each vascular risk factor, with hypertension being the primary cardiovascular risk. Studies show that antihypertensive medications targeting the renin angiotensin system lower the incidence and progression to AD1. Particularly, the angiotensin II (AngII) type 1 receptor (AT1R) antagonist losartan has been shown to restore cerebrovascular and cognitive deficits in AD mouse models2. Angiotensin IV receptors (AT4Rs) have been implicated in losartan's benefits3, but whether AT4Rs act alone or with AngII type 2 receptors (AT2Rs) is unclear. We investigated whether AT2Rs mediate benefits of chronic losartan treatment and whether chronic administration of an AT2R agonist could mimic losartan's benefits in AD transgenic mice overexpressing the Swedish and Indiana mutations of the human amyloid precursor protein (APP mice, line 20).

Methods: Wild-type and APP mice (2-3 months old) received losartan (10 mg/kg/day, 7 months) in their drinking water. Losartan treated mice received intracerebroventricular (icv) administration of CSF (control) or AT2R antagonist PD123319 (1.6 nmol/day) via osmotic minipumps. A subset of untreated mice received 1 month of compound 21 (1 nmol/day, C21, AT2R agonist, icv) via osmotic minipumps, while a separate cohort of mice was treated with C21 (10 mg/kg/day) in their drinking water for 7 months. Data were analyzed by two-way ANOVAs (genotype and treatment as factors), followed by Newman-Keuls multiple comparisons tests, and expressed by mean ± SEM.

Results: PD123319 failed to counter losartan's cognitive benefits measured in the Morris water maze (MWM) and novel object recognition (NOR) tests. C21 treatment had no benefit on any memory parameter. PD123319 countered losartan's ability to rescue sensory-evoked neurovascular coupling while C21 normalized this response in APP mice.

Losartan's benefits on nitric oxide (NO) bioavailability was reduced by PD123319 administration, while C21 improved NO bioavailability. Neither PD123319 or C21 treatments altered endothelial- or smooth muscle dilatory function.

Conclusions: AT2Rs contribute to neurovascular coupling and NO bioavailability benefits following chronic AT1R blockade in APP mice. Since AT2Rs failed to counter and AT2Rs agonism failed to mimic losartan's cognitive benefits, our results suggest that ATR2 may not be a fully effective therapeutic approach for AD. We conclude that the decreased incidence of AD in hypertensive patients treated by angiotensin receptor blockers4 may be related to AT4R rather than ATR2 activation.

References: 1. Khachaturian *et al*, Arch Neurol, 2006, 686-692 ; 2. Ongali B *et al*, Neurobiol Dis, 2014, 126-136; 3. Royea J *et al*, J Neuroscience, 2017, 5562-5573; 4. Wolozin B *et al*, Alzheimers Dement, 2008, 182-185.

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Searching for targets in post-stroke cognitive impairment: aberrant hippocampal neurogenesis

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Questions: Stroke-induced cerebrovascular injury is a major cause of neurological disability and dementia. Specifically, the prevalence of post-stroke cognitive impairment is about 15-40% among stroke survivors, depending on the type of patients included. Despite its importance, there is no specific treatment for this condition. This could be partly due to the relative paucity of experimental studies on the mechanisms involved and on the potential therapeutic targets. Therefore, our aim was to characterise cognitive deficits and the possible involvement of neurogenesis after stroke in mice.

Methods: Stroke was induced by permanent distal middle cerebral artery occlusion (MCAO) in 8-10 weeks-old C57Bl/6 mice. Cognitive deficits were assessed using contextual fear conditioning (CFC) and Barnes maze tests. Immunofluorescence was used for histological studies of neurogenesis. Comparisons between 2 groups and among >2 groups were performed with non-parametric Mann-Whitney test and with one-way or two-way ANOVA followed by Bonferroni post hoc testing, respectively. Differences were considered statistically significant at $p<0.05$.

Results: MCAO-exposed animals displayed impaired contextual and spatial memory performance, in parallel to a bilateral hippocampal neurogenesis burst that was maintained one month after stroke. Furthermore, stroke-induced newborn neurons promoted an aberrant hippocampal circuitry remodelling with differential features at ipsi- and contralesional levels. Remarkably, inhibition of stroke-induced hippocampal neurogenesis by temozolomide treatment or using a genetic approach (NestinCreERT2-NSE/DTA mice) impeded the forgetting of old memories.

Conclusions: Our results suggest that hippocampal neurogenesis modulation could be considered as a potential approach for post-stroke cognitive impairment.

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Glatiramer Acetate reduces infarct volume in diabetic mice with cerebral ischemia and prevents long term memory loss

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Questions: Stroke is currently the leading cause of acquired disability and the second cause of dementia after Alzheimer disease in industrial countries. The number of treated patients at the acute phase is low, due to the narrow therapeutic window. Diabetes is an independent risk factor that triples the incidence of stroke and worsens subsequent disability and cognitive decline. The growing pandemic of diabetes is thus a serious health challenge. We have previously shown that increased stroke severity in diabetes is partly due to the underlying microangiopathy (POITTEVIN et al., Diabetes 2015), associated with an increased pro-inflammatory response to ischemia (*unpublished data*). We hypothesize that switching from a pro-inflammatory towards an anti-inflammatory profile through the use of Glatiramer Acetate (GA), an immunomodulatory treatment of multiple sclerosis, will limit lesion expansion and prevent post-stroke dementia.

Methods: Diabetic (streptozotocin IP) male C57Bl6 mice were subjected to permanent middle cerebral artery occlusion. Mice received 2mg of GA or saline solution subcutaneously, administrated daily until 7 days post-stroke (D7). Sensorimotor evaluation was assessed at D1, D3, D7 and D42 post-stroke (HADDAD et al., Eur J Pharmacol 2008) and spatial memory (Barnes maze) from D29 to D38. Infarct volumes were measured at D3 and D7. Neurogenesis and microglia density were assessed at D3 and D7. Cytokines profile was assessed by RT-PCR and multiplex immunoassay. Statistical analyses were performed using t-tests and two-way Anova with repeated measures for long-term behavior analysis.

Results: In diabetic mice, GA decreased the infarct volume by 30% at D3, compared to saline (11.78±1.60mm3, n=7 vs 16.37±1.99mm3, n=7; p=0.018) and by 40% at D7 (2.59±1.27mm3, n=7 vs 6.47±4.2mm3, n=5; p=0.048). A better sensorimotor score was observed in GA treated mice at D3 (14±1.35, n=14 vs 11±3.55, n=11; p=0.013), but both GA treated and control mice had recovered by D7. Long-term memory loss was prevented (GA vs vehicle $p<0.05$* and NS vs D and C groups). Neurogenesis (Ki67+/Dcx+ cells in the ipsilateral hemisphere) was increased from D7 in GA treated mice (101.0±14.80, n=7 vs 78.50±10.93, n=5; p=0.034). GA did not modify significantly microglia cell density at D3 nor D7. No modification was observed concerning the cytokines inflammatory profile in GA treated mice, except at D3 where COX2 was decreased (0.40±0.14, n=7 vs 1.82±1.62, n=8; p=0.026) and at D7 for TNFα (3.316±8.01, n=5 vs 36.69±28.16, n=6; p=0.012).

Conclusion: Glatiramer acetate administered for 7 consecutive days post-stroke in diabetic mice reduced significantly the infarct volume, accelerated sensorimotor recovery, prevented long term memory loss and promoted neurogenesis, associated with decrease in COX2 and TNFα expression at D3 and D7, respectively

Cerebrovascular Changes in a Comorbidity Model of Vascular Dementia

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Introduction: Vascular contributions to cognitive impairment and dementia (VCID) is the second leading cause of dementia behind Alzheimer"s disease (AD). This broad term encompasses a spectrum of initial asymptomatic cerebrovascular changes (seen in small vessel disease and cerebral amyloid angiopathy where pathologic Amyloid Beta (Aβ)1-42 protein accumulates around brain blood vessels) to the profound symptomatic damage following acute stroke(s). Cerebrovascular remodeling and angiogenesis may represent early compensatory changes to the reduced blood flow seen in VCID by increasing the proteolytic turnover of the surrounding extracellular matrix. We

have demonstrated that one such extracellular matrix protein, perlecan (a heparan sulfate proteoglycan), possesses a C terminal domain V (DV) protein that upon cleavage from perlecan greatly enhances brain angiogenesis.

Questions: Our objective is to determine the cerebrovascular changes in a comorbidity mouse model of VCID that are associated with cognitive decline and increased mortality.

Methods: In our distinct mouse model (diabetic APP/PS1 knock in (db/AD)) of VCID, that is known to have gradual cognitive decline by 9 months with associated brain microangiopathy, Aβ deposition, increased mortality rates, and microhemorrhages, we evaluated gross dorsal and ventral cerebrovascular changes with a perfused vascular dye, Di I. We quantified microscopic cerebrovasculature protein changes (Collagen IV, PECAM, DV, and claudin-5) that can influence vascular integrity during the compensatory vascular remodeling and new blood vessel grown (angiogenesis) that we and others believe innately initially occurs to combat vascular degeneration and reduced blood flow in VCID.

Results: In db/AD animals (3-6 months), we observed a decrease in BBB proteins (i.e. claudin-5), indicating that altered cerebrovascular function that correlates with an increase in DV expression during the asymptomatic angiogenic stage, which precedes cognitive changes (9-12 months). Altered cerebrovascular protein expression in PECAM and Collagen IV correlate to aberrant two-photon vascular imaging, increased saccular aneurysms, and microhemorrhages.

Conclusions: Collectively, these data indicate increased expression of DV correlates to early cerebrovascular changes induce by angiogenic-remodeling that may compensate for cerebrovascular degeneration and reduces blood flood seen in VCID. In turn, the decline in DV expression is associated with aberrant vasculature and increased hemorrhages that contribute to increased mortality, suggesting that DV "replacement therapy" could represent a novel therapeutic for VCID.

Spatial memory deficits are concomitant to an impaired hippocampal neurogenesis in a mouse model of cerebral hypoperfusion

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Questions: Vascular cognitive impairment (VCI) is defined by alterations in cognition attributable to cerebrovascular causes that can range from subtle deficits to complete vascular dementia. One of the most important causes of VCI is chronic cerebral hypoperfusion (CCH), which produces white matter lesions triggered by oxidative stress, inflammation and blood-brain barrier damage (Iadecola, 2013). Several studies have linked a faulty neurogenesis with poor outcome after CCH in rats (Silvilia et al., 2008; Choi et al., 2016). However, the spatial memory deficits that underlie CCH and could be developed by a defective hippocampal neurogenesis have not been studied so far.

Methods: Ten-week-old male C57Bl/6 mice were subjected to bilateral common carotid artery stenosis (BCCAS) or sham operation. Hippocampus-dependent spatial memory deficits were evaluated 3 months after surgery using the Novel Object Location (NOL) and Y-maze tests. Neurogenesis was quantified at 1, 3, 7, 14, 28 days and 3 months by immunohistochemical studies. Data were analyzed by two-way ANOVA with Bonferroni post-test or t-test.

Results: Three months after surgery, BCCAS-animals showed spatial memory deficits demonstrated by a lower time in the closed arm of the Y-maze and lower exploration time of the novel object than their sham-operated counterparts (recognition index: sham 2.21 ± 0.23 *vs*. BCCAS 3 months 0.93 ± 0.17; n=6, $p<0.05$). Doublecortin-positive cells showed a significant decrease (sham 117.7 ± 3.13 vs. BCCAS 3 months 93.04 ± 6.2 cells; n=6, $p<0.05$) in mice subjected to BCCAS with no differences in progenitor proliferation measured as Ki67+ cells (n=6, $p>0.05$).

Conclusions: CCH in rodents produces deficits in hippocampus-dependent spatial memory in the short and long term which are concomitant to a decrease in the number of neuroblasts, suggesting that altered neurogenesis accounts for cognitive impairment in this model.

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CADASIL brain vessels show a HTRA1 loss-of-function profile

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Introduction: CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) and the phenotypically related recessive condition CARASIL have emerged as important genetic model diseases for studying the molecular pathomechanisms of cerebral small vessel disease (SVD). Both syndromes show considerable overlap in clinical and histopathological features including early-onset and recurrent ischemic strokes, extensive white matter hyperintensities and a pronounced vasculopathy of small and medium-sized brain vessels. Nevertheless, they represent distinct genetic diseases caused by neomorphic NOTCH3 mutations and loss-of-function mutations in the high temperature requirement protein A1 (HTRA1) protease respectively. Unique features of CADASIL are the aggregation of the Notch3 extracellular domain ($Notch3^{ECD}$) and the formation of vascular protein deposits of insufficiently determined composition, the probable starting point of a pathological cascade resulting in vessel dysfunction.

Methods: We determined the brain vessel proteome from autopsy material of CADASIL patients and controls (n = 6 for each group) by quantitative mass spectrometry. Immunohistochemistry and immunoblotting were used to verify accumulation and localization of individual proteins. Proteomic data were compared with the brain vessel proteome of HTRA1 knockout mice. *In vitro* protease activity assays were conducted to assess HTRA1-mediated processing of putative substrates.

Results: The proteomic analysis of CADASIL vessels revealed a clear tendency towards protein accumulation and identified 95 primarily secreted proteins with significantly increased abundance. HTRA1 was found to be strongly enriched (4.9-fold, $p = 1.6 \times 10^{-3}$) and shown to colocalize with $Notch3^{ECD}$ deposits in patient vessels suggesting a sequestration process. Moreover, the observed accumulation of several HTRA1 substrates was compatible with their reduced degradation as a consequence of HTRA1 inactivation. Comparison of the proteomic data with the brain vessel proteome of HTRA1 knockout mice revealed a highly significant overlap of 18 enriched proteins ($p = 2.2 \times 10^{-16}$), several of which were identified as novel substrates by an *in vitro* proteolysis assay.

Conclusion: Our study represents the most comprehensive proteomic analysis of CADASIL-affected vessels to date providing an in-depth view of disease-relevant protein abundance alterations. The recruitment of the HTRA1 protease to pathological $Notch3^{ECD}$ deposits and its inactivation might be critical steps in CADASIL pathogenesis linking the molecular mechanisms of two distinct SVD forms.

Neuroinflammation I

Immunological mechanisms of stroke-derived immunosuppression

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Background: Acute brain lesions induce a multiphasic peripheral immune response. Acute activation of peripheral immune cells is followed by subacute immunosuppression. This phenomenon can lead to post-stroke infections which account for almost 20 % of in-hospital deaths and poor outcomes. However, the mechanisms causing such opposing immune alterations are barely understood. In this study, we investigated the soluble mediator-derived activation of the inflammasome and subsequent pyroptotic cell death as a potential explanation for subacute immune exhaustion and stroke-induced T cell death.

Methods: Acute brain ischemia was induced by transient middle cerebral artery occlusion (MCAO) in WT, RAGE $^{-/-}$, MyD88$^{-/-}$, Casp-1$^{-/-}$ and ASC$^{-/-}$ mice. Immune cell populations and caspase-1 activation were analysed by flow cytometry after stroke. Moreover, analysis of caspase-1 activation via Western blot was performed. The role of soluble mediators was investigated in a mouse model of parabiosis. For confirmation *in vitro*, a live cell imaging setup with bone marrow-derived macrophages (BMDMs) and T cells was used.

Results: Already 18 h after stroke a severe decrease of CD45^{+}CD3^{+} T cell numbers in spleen (50±5 %) and whole blood (60±10 %) was found compared to sham-operated mice. Analysis of whole splenocytes via Western blot revealed an increase in cleaved (activated) caspase-1 levels 12 h after stroke compared to sham and naïve mice, indicating inflammasome activation after stroke. Subsequently, we used global Casp-1$^{-/-}$ mice and found less lymphopenia after stroke compared to WT mice. To identify if the activated inflammasome in T cells itself leads to cell death after stroke we transferred Casp-1$^{-/-}$ or ASC$^{-/-}$ T cells in lymphocyte-deficient (Rag-1$^{-/-}$) mice. No improvement in T cell death was found compared to transferred WT T cells, indicating no T cell-autonomous inflammasome-derived cell death. Further analysis of splenic monocytes via flow cytometry revealed increased levels of active caspase-1 after stroke. We used a parabiosis model for identification if soluble mediators activate the inflammasome and found a decrease of T cells in blood and spleen not only in the ischemic parabionts but also in the non-operated mice. In order to confirm the hypothesis of soluble mediator-induced cell death, T cells as well as BMDMs were cultured and then treated with sham or stroke serum. BMDMs and T cells died, when treated with stroke serum. However, BMDM cell death was reduced when treated with denatured or RNase/DNase-treated stroke serum. Additionally, lymphopenia was reduced in pattern recognition receptor-KO mice (RAGE $^{-/-}$ and MyD88$^{-/-}$), suggesting that soluble mediators induced post-stroke T cell death via activation of the monocytic inflammasome.

Conclusion: This study provides first evidence that monocytic inflammasome activation leads to fatal post-stroke T cell death and subsequently to an immunosuppressive phenotype giving rise to secondary infections.

Gene expression signatures of dendritic cells and microglia in the ischemic brain tissue of mice

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Questions: Dendritic cells (DCs) are the professional antigen presenting cells (APCs) that migrate from secondary lymphoid organs into inflamed tissues. Seminal studies reported the presence of APCs in the ischemic brain tissue and identified some of these as infiltrating DCs. However, microglial cells can also present antigen and the phenotypic and functional differences between reactive microglia and DCs in the ischemic tissue are not well defined. We hypothesise that DCs are different from microglia and exert distinct functions in the injured brain tissue.

The objectives of this study were: a) to identify differences in the gene expression profile of DCs and microglia in the ischemic brain tissue, and b) distinguish infiltrating DCs from APCs originating from brain resident cells.

Methods: We induced ischemia in adult mice (background C57BL/6J) by 45-min middle cerebral artery occlusion (MCAo, filament model) and studied the brain tissue 4 days later. We used DC reporter mice expressing eYFP under the promoter of CD11c, from which we sorted by FACS the eYFP$^+$ cells both from the ischemic brain tissue and from the spleen. We also sorted microglia from control and ischemic brain hemispheres of microglia reporter mice generated by crossing CX3CR1-creERTmice with B6.Cg-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J mice. These latter mice express a red fluorescent protein in CX3CR1$^+$ microglial cells after administration of tamoxifen. RNA was extracted from the sorted cells and was studied by RNAseq. We identified infiltrating DCs by generating parabiotic mice with CD11c-eYFP and littermate wild type (wt) mice. The wt mouse of each parabiotic pair was subjected to MCAo and the brain was obtained 4 days later for immunofluorescence, flow cytometry or RNAseq studies of eYFP$^+$ infiltrated cells.

Results: We identified genes differentially expressed (LogFC >|2|, p value <0.001) in DCs vs. microglia of the ischemic brain tissue, and we also examined the differences between DCs obtained from the brain and the spleen. Genes related to antigen presentation were overrepresented in DCs compared to microglia and genes typically expressed in microglia showed no or very low expression in DCs, suggesting that DCs are indeed a distinct population of cells. Parabiosis resulted in about 40-50% of eYFP$^+$ spleen DCs in the wt mouse of each parabiotic pair. We observed eYFP$^+$ cells in the ischemic brain tissue, but not in the contralateral hemisphere of the parabiotic wt mice, demonstrating the infiltration of peripheral DCs. We are currently comparing the gene expression profile of eYFP$^+$ cells obtained from the ischemic brain tissue of parabiotic mice versus that of CD11c-eYFP mice.

Conclusions: This study shows that DCs infiltrate from the periphery to the lesioned tissue and have a distinct gene expression signature, which differs from that of microglia in the ischemic brain tissue.

Surgical stress alters the intestinal immune system in the mouse model of cerebral ischemia/reperfusion

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Questions: The nervous system is functionally and anatomically connected to the immune system. Acute stroke and other forms of stress induce neuro-hormonal responses affecting the immune system. Several lines of evidence support stroke-induced alterations of gut immune cells that in turn exacerbate the brain inflammatory response. Experimental stroke models require surgery and deep anesthesia that do not take place in stroke patients. Both anesthetic and surgical stress affect systemic inflammation and leukocyte populations in the bone marrow. The objective of this study was to identify stroke-induced alterations in gut immune cells by specifically monitoring the changes induced by surgery and anesthesia in appropriate sham conditions.

Methods: We induced ischemia in adult male mice on the C57BL/6J background by 45-min middle cerebral artery occlusion (filament model) and 48h reperfusion. To control for the effects of experimental surgery and anesthesia (90 min in total) we conducted in parallel sham-operations, where we followed all the procedures excepting placement of the filament. We also studied control mice devoid of any intervention. We dissected the gut and quantified lymphocyte subset number and frequency of cells in the intestinal mucosal immune system by flow cytometry. We validated the success of tissue separation by specific hallmarks, as follows: Peyer"s patch (PP) cells had a high relative frequency of B cells in comparison with the mesenteric lymph nodes (mLN); intraepithelial lymphocytes (IEL) were identified by their total presence of CD103+CD69+ resident memory T cells (Trm); lamina

propria (LP) leukocytes contain CD11b+ myeloid cells in addition to lymphocytes. IELs and LP cells were assessed from both small intestine (SI) and colon.

Results: We obtained consistent and uniform leukocyte populations for each regions. IEL cell number was drastically reduced after stroke vs. controls. However, the main causative mechanism was related to surgical stress because the same response was found in sham-operated mice. A similar effect was observed in mLN with some cellular specificity. Whereas T cells progressively decreased in sham-operated and ischemic mice, B cells soared only in sham mice. These cells showed particular changes in the SI where their number decrease in IEL and conversely increase in LP in response to surgical stress. Treg cells responded similarly to B cells.

Conclusions: Models of experimental stroke introduce artifactual alterations of the gut immune system due to anesthesia and surgical stress that are not mediated by specific immune response to stroke. Improved methodologies and/or strict control of non-stroke related technical aspects are critical to improve the success of stroke translational studies.

Chronic post-stroke T cell responses affect functional recovery

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Question: T cells are recruited to the ischemic brain as early as 24hrs post-stroke and exhibit diverse protective and detrimental effects in the acute phase. Recent studies show persistence of immune cells in the chronic phase following stroke and T cell egress into the post-stroke brain might actually peak weeks after onset, suggesting a long-term role for the adaptive immune system in the injured CNS. Moreover, stroke patients with increased antigen presentation of the neuronal antigens MAP2 and NMDA receptor subunit 2A (GluN2A) exhibited smaller infarctions at day 7 and better long-term improvement at 3 months. We therefore hypothesized that post-stroke T cell responses have a neuroprotective profile and are potentially CNS-specific responses, affecting long-term functional recovery.

Methods: Male C57BL/6 mice (8-10 weeks) were subjected to 60min transient middle cerebral artery occlusion (tMCAo). Spleens were harvested 8 and 10 days post-stroke, stained with CFSE, and cultured with GluN2A and MAP2 peptides re-stimulation for 6 days. Cell cultures were stained with fluorescently-tagged antibodies and quantified by flow cytometry. Another cohort of mice were euthanized 14 days and 30 days after stroke surgeries. Immune cell subpopulations in the brain were quantified using flow cytometry, infarct volume was determined using MRI, and functional recovery was quantified using rotarod behavioral test.

Results: Post-stroke mice had increased numbers of CD4 T cells and CD8 T cells in their ischemic hemisphere compared to contralateral hemisphere at 14 days (19-fold, $p<0.0001$ and 8-fold, $p=0.0084$) and 30 days (8-fold, $p=0.0031$ and 7-fold, $p=0.0013$) after stroke, respectively. At 30 days post-stroke, 35% of CD4 T cells and 68% of CD8 T cells in the ischemic hemisphere were IFNγ positive. Mice with higher numbers of CD4 T cells ($R2=0.33$, $p=0.0525$) and CD8 T cells ($R2=0.52$, $p=0.0082$) in ipsilateral hemisphere had worse functional recovery in the rotarod behavioral test. Antibody-mediated depletion of CD8 T cells in the periphery at 10 days post-stroke, depleted these cells in the brain as well. Moreover, percentage of brain infiltrating post-stroke CD45+ immune cells were 2-fold higher in CD8 T cells depleted mice, compared to control ($p=0.0083$). Stroke induced higher splenic CNS-autoreactive T cells compared to sham surgery (10/12 vs 4/7 mice). CD8 T cells (10/12 mice) had higher GluN2A-specific responses compared to CD4 T cells (4/12 mice).

Conclusions: Our data show that there are long-term T cell responses in the ischemic hemisphere post-stroke. These chronic phase T cells affect immune cell numbers present in the brain long-term and the functional recovery post-stroke, potentially through IFNγ signaling. We also describe novel neuronal antigen-specific T cells responses in the

spleen post-stroke. In summary, our findings suggest that T cell responses play a significant role in functional recovery post-stroke beyond the first week after onset.

Phagocytosis in the ischemic brain assessed by superresolution structured illumination microscopy

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Questions: Phagocytosis is a key function of myeloid cells and is highly involved in brain ischemic injury. It has been scarcely studied *in vivo*, thus preventing a deep knowledge of the processes occurring in the ischemic environment. Structured illumination microscopy (SIM) is a superresolution technique. It may be used to study phagocytosis, which involves vesicles sized below the resolution limits of standard confocal microscopy. We here use SIM to measure phagocytosis after cerebral ischemia in tissue specimens of ischemic mice.

Methods: Mice underwent permanent occlusion of the middle cerebral artery and were sacrificed at 48h or 7d after insult. Immunofluorescence for CD11b, myeloid cell membrane marker, and CD68, lysosomal marker was done in the ischemic area. Images were acquired using a SIM system by Nikon and verified with SIM check. Lysosomal distribution was measured in the ischemic area by the gray level co-occurrence matrix (GLCM). SIM dataset was compared with transmission electron microscopy images of macrophages in the ischemic tissue at the same time points. Cultured microglia were stimulated with LPS to uptake 100 nm fluorescent beads and imaged by time-lapse SIM. GLCM was used to analyze bead distribution over the cytoplasm. Statistical power was assessed pre-hoc using pilot study. Groups were compared using t-test or Two-way ANOVA followed by an appropriate *post hoc* test.

Results: SIM images reached a resolution of 130 nm and passed the quality control diagnose, ruling out possible artifacts. After ischemia, GLCM applied to the CD68 images showed that, myeloid cells at 48h had higher angular second moment (ASM, 0.60 ± 0.19, mean ± sd), inverse difference moment (IDM, 0.82 ± 0.09) and lower entropy (2.14 ± 1.02) than myeloid cells at 7d (ASM: 0.39 ± 0.18, IDM: 0.73 ± 0.08, entropy: 3.22 ± 0.92) indicating higher lysosomal clustering at 48h. At this time point lysosomal clustering was proximal (< 700 nm) to the cell membrane indicating active target internalization, while at 7d it was perinuclear, consistent with final stages of phagocytosis or autophagy. Electron microscopy images indicated a similar pattern of lysosomal distribution thus validating the SIM dataset. GLCM on time lapse SIM from phagocytic microglia cultures revealed a temporal decrease in ASM and IDM and increase in entropy, as beads were uptaken, indicating that GLCM informs on the progression of phagocytosis.

Conclusions: GLCM analysis on SIM dataset quantitatively described different phases of macrophage phagocytic behavior revealing the dynamics of lysosomal movements in the ischemic brain indicating initial active internalization vs. final digestion/autophagy.

Cytokines associated with neuroinflammation alter extracellular vesicle production and cargo, which enhance mitochondrial function in recipient cells

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Question: Extracellular vesicles (EVs) are small (40 – 1000 nm) membrane-bound vesicles released from most, if not all cell types. EVs can carry functionally active cargo (proteins, nucleic acids) that can be taken up by neighboring cells and mediate physiologically relevant effects. In this capacity, EVs are being regarded as novel cell-to-cell communicators, and may play important roles in the progression of neuroinflammatory diseases. By spreading pathological proteins or RNAs, EVs may transfer deleterious molecules to healthy cells, thereby enhancing inflammation or causing further cell death. In the current study, we investigated how various cytokines shown to be upregulated during neuroinflammation affect EV production, microRNA profiles, and mitochondrial function in a neuronal cell line. Further, we investigated the effects of cytokine-induced EVs on naïve recipient cells.

Methods: After a 24 hour exposure period of HT-22 cells to TNF-α or IFN-γ, EVs were isolated from the conditioned media and their size distribution was profiled using the NanoSight NS300. Using qRT-PCR we then profiled the EV and intracellular levels of three miRs associated with neuroinflammation. Several mRNA targets of these miRs include proteins involved in energy metabolism, so mitochondrial function and cell viability were also assessed using the Seahorse Bioanalyzer, and LDH respectively. In addition, we exposed naïve cells to cytokine-induced EVs and measured mitochondrial function and cell viability. Further, we explored the role of vesicular transfer of cAMP, with subsequent PKA inhibition studies.

Results: Exposure to either cytokine significantly increased EV secretion compared to control. Exposure to TNF-α induced a dose-dependent increase in all three miRs contained within EVs. IFN-γ did not alter EV or cellular miR profiles. Exposure to either cytokine did not induce mitochondrial dysfunction, however, exposing naïve cells to isolated cytokine-induced EVs significantly increases mitochondrial respiratory capacity and ATP production. Further, we have detected the presence of cAMP in EVs and believe mitochondrial enhancement is being mediated by vesicular transfer of cAMP, subsequent PKA activation in recipient cells, followed by downstream phosphorylation of electron transport chain proteins. Thus, we then exposed cells to cytokine-derived EVs in the presence of a PKA inhibitor, which eliminated EV-induced mitochondrial enhancement.

Conclusions: These data suggest that various cytokines can induce EV secretion, yet give rise to differential EV populations. Differences in EV and intercellular miR profiles suggest the potential of sequence-specific packaging and secretion mechanisms. Although the miR content of the cytokine-induced EVs would theoretically induce mitochondrial dysfunction, here we observe significant enhancement in respiratory capacity and ATP production, indicating that another mechanism is likely dominating.

TREM2 controls subacute CNS myeloid cell accumulation and reactivity and promotes injury resolution and functional recovery after stroke

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Introduction / question: Understanding how the brain remodels and adapts to damage caused by stroke is essential to develop alternative therapeutic approaches that can be applied over a greater timeframe than existing hyperacute treatments. Growing evidence suggests that specific subsets and phenotypes of microglia and CNS macrophages can support tissue repair and recovery of function. Understanding the endogenous mechanisms controlling these phenotypes and identifying exogenous approaches to direct CNS myeloid cells towards pro-resolving and pro-regenerative properties after stroke will be important for developing new therapies. We have investigated the involvement of TREM2, a cell surface immunomodulatory receptor expressed on myeloid cells, in injury resolution, brain repair and functional recovery using a model of experimental stroke in mice.

Methods: Experimental stroke was induced in wild-type or TREM2-/- mice using the permanent middle cerebral artery occlusion method. At various timepoints during the acute, subacute and chronic phases, a range of histological, molecular and functional analyses were performed. Chimaeric mice were generated with TREM2 expression restricted to microglia to enable the contribution of TREM2 in different myeloid subsets to be determined.

Results: TREM2 expression was induced in the subacute phase after stroke. Reactive microglia and macrophages accumulated around the peri-infarct border however this was suppressed in TREM2-/- mice and significantly fewer microglia and monocyte-derived macrophages (CCR2+ and CCR2-) were present in the injured hemisphere of TREM2-/- mice. Subacute injury resolution and functional recovery were also impaired in TREM2-/- mice and chimaeric studies showed this was dependent on microglial-derived TREM2 expression. The induction of transcriptional networks controlling myeloid cell activation, angiogenesis and matrix remodelling were blunted in TREM2-/- mice.

Conclusions: Our findings emphasise the importance of appropriate myeloid cell reactivity to promote injury-induced brain remodelling and subacute functional recovery and demonstrate TREM2 as a key endogenous regulator. Developing approaches to harness/augment TREM2-driven pathways and more generally pro-regenerative CNS myeloid cell activity may offer new opportunities to treat patients in the chronic phase after stroke.

Cell-based therapies

In stroke, functional connectivity is stabilised by stem cell graft but structural network responds only to ischemic lesion

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Introduction: In recent years, investigations on stroke have broadened the attention from a mere focus on the immediate ischemic effects towards inclusion of the whole brain with emphasis on the long range effects, distal to ischemic tissue focus. Outcome analysis of stem cell therapies also requires the understanding of the graft"s influence on the whole-brain networks. Here, we have longitudinally and noninvasively monitored the structural and functional network alterations in the mouse model of focal cerebral ischemia during spontaneous development and after stem cell implantation.

Methods: Nude mice were submitted to 30 min MCA occlusion with the intraluminal filament model. Human neural stem cells, transduced to constitutively express luciferase, were implanted into the cortex, close to the ischemic territory. Bioluminescence imaging was performed for vitality imaging of the graft. Resting state fMRI was recorded at 9.4T to determine the functional sensorimotor networks and structural networks were determined with diffusion-sensitive Q-ball imaging. All imaging modalities were repetitively performed to compile a temporal profile over 12 weeks post stroke induction.

Results: In corticostriatal lesions, the functional sensorimotor network was substantially weakened including even transhemispheric functional connections at 2 weeks, and persisting for the whole 12 weeks period. In the group with stem cells grafting 2 days after stroke, the functional network was completely stabilised at 2 weeks, comparable to the pre-ischemia condition. Between 4 and 6 weeks, the functional connectivity weakened in this group, too, approximating the situation of the untreated stroke group thereafter. Vitality monitoring by bioluminescence imaging of the graft presented a clear reduction of graft vitality after 4 weeks. Structural connections showed decreases of fiber density and some increases in fiber density between sensorimotor cortex and white matter regions belonging to the CST. All structural changes were induced by the ischemic lesion in comparable amount in both groups. When stem cells were grafted into cortex of mice with only striatal lesion, graft vitality remained stable during the 12 weeks and functional connectivity also persisted at pre-stroke, normal level.

Conclusion: Structural changes of fiber-density increases are stimulated by the endogenous tissue without further modulation by the stem cells, while functional networks are stabilised by the stem cells via a paracrine effect. Persistence of such functional networks stabilisation is based on a viable graft at quantitative scale. These results will help decipher the underlying mechanisms of brain plasticity in response to stem cell treatment.

Multiple autologous bone marrow-derived 271+ mesenchymal stem cells transplantations overcome drug resistant epilepsy in children - a six year study

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Background: Patients suffering from drug resistant epilepsy (DRE) are still waiting for more efficient and less toxic treatment. Recent advances in a field of cell therapy bring new options for those patients. The objective of present study was to assess the safety, feasibility, and potential efficacy of multiple autologous bone marrow cells transplantations in pediatric DRE patients.

Methods: 19 children with DRE were enrolled into the study. All children undergone combined autologous cell therapy of single bone marrow nucleated cells (BMNCs) transplantation and four rounds of bone marrow mesenchymal stem cells (BMMSCs) transplantations. The BMMSCs used in the study were a unique population derived from CD271 positive cells. Intrathecal transplantations were performed directly into cerebro – spinal fluid (CSF). Neurological evaluation included magnetic resonance imaging (MRI), electroencephalography (EEG) and cognitive development assessment using neuropsychological tests. Properties of patient"s BMMSCs were also evaluated.

Results: Intravenous and intrathecal transplantations were performed causing no adverse events, showing safety and feasibility during 4 - years follow-up. Importantly, the therapy caused neurological and cognitive improvement in all patients: significant reduction in a number of epileptic seizures (from initial 10/day to 1/week) and absence of SE episodes (from 4/week to 0/week). The therapy decreased number of discharges in EEG evaluation and caused cognitive improvement in the sphere of reaction to light and sound, in the sphere of emotions and in the sphere of motor function. Analysis of BMMSCs properties revealed expression of neurotrophic, proangiogenic and tissue remodeling factors. Their immunomodulatory potential was also shown.

Conclusions: Our results demonstrate the safety and feasibility of BMNCs and BMMSCs transplantations in children with DRE. Moreover, the results demonstrate that cell therapy approach brings considerable neurological and cognitive improvement for those patients.

Neurons derived from embryonic stem cells extend projections into lesioned brain and promote functional recovery after stroke

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Question: Ischemic injuries within the motor cortex result in functional deficits that may profoundly impact activities of daily living in patients. Transplantation of stem cells has been employed to improve functional recovery after ischemic damage. Recent studies have shown that murine embryonic stem cells (mESC) derived neurons transplanted into the adult brain can integrate into the host tissue, send long distance projections and make functional synapses. A crucial goal in cell replacement protocols is the ability to produce the wanted type of neural cell to be replaced. Our group has recently developed a specific differentiation protocol that allows to produce either hippocampal or cortical neurons from mESCs. Here we compared the ability of these cells to generate long range projections in a region-specific manner after transplantation in the healthy or damaged brain. We also studied the impact of cell grafting on motor recovery after cortical ischemic lesion.

Methods: Cortical and hippocampal mESC derived neurons, labelled with lentiviral vectors carrying two different membrane bound fluorophores, have been co-injected in the hippocampus and primary motor cortex of adult

healthy mice by using a micropump. Then, the integration and projection patterns of these cells have been analyzed in a mouse model of ischemic lesion induced by Rose-Bengal mediated photothrombosis in the primary motor cortex. In this case, cells are injected three days after stroke and the assessment of recovery is based on well established motor tasks (Schallert Cylinder test and Gridwalk Test) (Spalletti et al, 2014). Four-eight weeks after the grafting, the survival and projection patterns of cortical and hippocampal mESC-derived neurons have been evaluated by histological analysis.

Results: Our data show that both hippocampal and cortical mESC-derived neurons are able to survive several weeks in the host tissue. Specifically, hippocampal-like cells transplanted in the hippocampus sent specific projections to the CA3 area and were able to elongate fibers even when transplanted in the motor cortex, independent of the presence of the ischemic lesion. In contrast, cortical-like cells showed a remarkably different projection patterns in healthy and stroke animals. In particular, the quantification of fibers demonstrate that cortical-like neurons transplanted in ischemic lesion display a higher number of projections with respect to the healthy, naïve brain. Importantly, the motor tasks showed evidence for behavioral recovery after stroke and transplantation of the cortical-like cells.

Conclusions: In this study, we demonstrate that neural precursor cells carry intrinsic signals regulating their axonal extension in different regions, and that the damaged adult brain provides signals supporting axonal projections by cortical cells. Importantly, grafting cortical cells promoted functional restoration of forelimb function after ischemic damage to the motor cortex.

Investigating the Role of the Mesenchymal Stem Cell Secretome in Promoting Repair after Ischaemic Stroke

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Questions: Mesenchymal stem cells (MSCs) hold great potential as a therapy for stroke and have previously been shown to promote recovery in preclinical models of cerebral ischaemia. MSCs secrete a wide range of growth factors, chemokines, cytokines and extracellular vesicles - collectively termed the secretome. The MSC secretome has been implicated in promoting repair through a number of mechanisms including decreasing inflammation, preventing cell apoptosis and by promoting endogenous repair mechanisms such as neurogenesis and angiogenesis. In this study we assessed the efficacy of the MSC-derived secretome (conditioned medium) on functional recovery, neuroinflammation and lesion volume in a mouse model of focal cerebral ischaemia.

Methods: Passage 5-6 human bone marrow-derived MSCs from a 22-week old fetal donor (3H Biomedical, Sweden) were used for all experiments. MSCs were primed with 10 ng/ml human recombinant IL-1α for 5 minutes and conditioned medium was collected, filtered and 10x concentrated at 24 h. Stroke was induced in 12-16 week old male C57BL/6 mice (Charles River Laboratories, UK) using the intraluminal filament model of middle cerebral artery occlusion. Either 400 µl 10x concentrated conditioned medium or MesenPRO (vehicle) was administered at the time of reperfusion by subcutaneous injection. Mice were then recovered for 14 days and underwent MRI scans at 48 h and a battery of behavioural tests to assess recovery.

Results: MSC-derived conditioned medium led to a ~30% reduction in lesion at 48 h and was associated with a modest improvement in functional recovery. Conditioned medium treated mice lost less body mass acutely and performed better in the nest building task day 3 post-stroke compared with vehicle treated animals. Conversely, there were no differences in neuroscore, accelerating rotarod and burrowing behaviour between conditioned medium and vehicle groups.

Conclusions: Our results suggest acute administration of MSC-derived conditioned medium had a neuroprotective effect and led to modest improvements in functional recovery after ischaemic stroke. Ongoing studies are investigating effects of the MSC secretome with delayed administration outwith the window for neuroprotection.

Patient-specific iPSC-derived neuroepithelial stem cells for neuroprotection and neuroregeneration in Machado-Joseph disease

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Machado-Joseph disease (MJD) is a neurodegenerative hereditary disease caused by an expanded polyglutamine tract within the Ataxin-3 protein. This mutant Ataxin-3 protein causes neuronal dysfunction and degeneration in specific brain regions, such as the cerebellum. We previously demonstrated that murine neural stem cells (NSC) transplantation in mice promote MJD-associated neuropathology and motor impairments improvement (Mendonça et al., Brain 2015). Nevertheless, most available human NSC sources are associated with ethical and immunological problems, potentially overcome by induced-pluripotent stem cells (iPSC)-derived neural progenitors.

Question: Is it possible to generate iPSC-derived neuroepithelial stem cells (NESC), from MJD-patients fibroblasts, adequate for cerebellar transplantation?

Methods: Human iPSC-derived NESC were obtained by reprogramming fibroblasts of Control (2 clones/cell lines) and MJD-patients (3 clones/cell lines), with lentivirus encoding for Oct-4, Klf4, c-Myc and Sox-2 into iPSC, which were induced to NESC. The obtained cell lines were tested for multipotency; ability to originate glia and mature functional neurons; neuronal neurite length and number of excitatory and inhibitory synapses and, exosomal content. Moreover, the NESC were transplanted into the cerebellum of NOD/SCID mice and cell viability, neuroinflammation and ability to differentiate into neural cells were assessed two months upon transplantation.

Results: Control and MJD patient-derived cell lines upon in vitro differentiation originated heterogeneous cultures composed by cells positive for glial (GFAP, S100B), neuronal (MAP2 and β3-tubulin), functional excitatory (VGlut and PSD95) and inhibitory (Gephyrin) synapse markers and, by cells responding to potassium but not to histamine stimulation, consistent with functional neurons profile. Preliminary results indicate that exosomes of iPSC-derived NESC of MJD-patients have different levels of autophagy and antioxidant enzyme markers. Finally, the control and MJD patient-derived NESC transplanted into mice´s cerebellum survived, migrated out of the transplantation site and differentiated into neurons and glial cells. No major neuroinflammation was detected and a minor co-localization between graft-derived cells and cell death markers was observed.

Conclusions: Our results indicate that it is possible to generate iPSC-derived NESC from fibroblasts of MJD-patients with potential to be tested as a cell source for neuroregeneration and neuroprotection.

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A dose-response *in vitro* of extracellular vesicles to evaluate their efficacy in an animal model of subcortical stroke

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Questions: *In vitro* study to evaluate the minimum effective dose of extracellular vesicles (EVs) that promote functional recovery in an animal model of subcortical stroke.

Methods: For *in vitro* studies of neurons and oligodendrocytes with different doses of EVs (50µg, 100µg, 200µg) we study cells viability (trypan blue) and neurogenesis (Doublecortin (DCX), MAP-2) and oligodendrogenesis ((Myelin Oligodendrocyte Glycoprotein(MOG)) expression markers by immunofluorescence after 72h. In the animal model, male and female Sprague-Dawley rats were submitted to a subcortical stroke (endothelin-1 injection). 24h after stroke we administrated EVs intravenously. Study groups (n=10 per group) were as follows: Control: subcortical stroke+saline; subcortical stroke+50µg EVs. We evaluated: motor function at 24h, 7 and 28days after stroke (Rogers, Rotarod and Beam Walking tests), lesion size and tract connectivity by magnetic resonance imaging at 24h and 28days post-stroke, cell proliferation (Ki-67), cell death (TUNEL), and brain repair markers: Glial Fibrillary Acidic Protein (GFAP), Synaptophysin (SYP), Brain-Derived Neurotrophic Factor (BDNF), Myelin Basic Protein (MBP), MOG and Vascular Endothelial Growth Factor (VEGF) by immunofluorescence at 28days after stroke. The data were compared using the Kruskal-Wallis test followed by the Mann-Whitney test.

Results: *In vitro* the dose of 50µg of EVs increased significantly cell viability and the expression of DCX, MAP-2 and MOG compared to the control, 100µg and 200µg groups ($p<0.05$). At 28days, animals treated with 50µg of EVs showed a significant improvement in all test of functional evaluation compared to the control group ($p<0.05$). At 28 days, we did not observe a reduction of the lesion size, however, we observe a significant increase in tract connectivity and cell proliferation, as well as a reduction of cell death in the animals treated with 50µg compared to the control ($p<0.05$). Finally, 50µg of EVs increased SYP, BDNF, MBP and MOG expression and decreased GFAP expression in the perilesional zone in comparison to the control group ($p<0.05$).

Conclusions: 50µg of EVs *in vitro* is the minimum effective dose that promotes cell viability and the expression of neurogenesis and oligodendrogenesis markers. In an animal model of subcortical stroke, 50µg of EVs improves functional recovery associated with protection and brain repair.

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Encapsulating Endothelial Progenitor Cell secretome in magnetized biocompatible nanocapsules for targeted cell-free therapy for neurorepair after stroke

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Introduction: Neurorepair treatments are being investigated for delayed phases of stroke with stem/progenitor cells-based therapies as promising strategies. However, we are facing two challenges: targeted brain-delivery and sustained effects during the recovery phase. Our aim is to investigate a new nanomedical product to deliver therapeutic endothelial progenitor cells (EPCs) secretome for angio-neurogenesis enhancement and demonstrate a safe local *in vivo* delivery in a mouse model of cerebral ischemia. For this we have encapsulated EPCs secretome in magnetized poly(D-L-lactic-co-glycolic acid) (PLGA) nanocapsules with superparamagnetic iron oxide nanoparticles (SPIONs) for its safe delivery under a magnetic field.

Methods: Secretome was obtained from human EPCs, cultured in multilayer flasks by collecting their secretome during 24 hours in basal media (EBM) which was concentrated, lyophilized and encapsulated into PLGA nanocapsules (230nm diameter) synthesized by a double emulsion-solvent evaporation method and embedded with SPIONs. *In vitro*, human cerebral microvascular endothelial cells (hCMEC/D3), neural stem cells (NSCs) obtained from C57BL/6 mice subventricular zones and differentiated oligodendrocyte precursor cells (OPCs) were treated with EPCs secretome and their respective EBM control. The effects were tested in proliferation assays and immunofluorescence. *In vivo*, mice received nanocapsules by intra-venous injection and followed during 2 weeks to assess their potential toxicity. For local brain targeting mice were subjected to intraluminal middle cerebral artery

occlusion, nanocapsules administered thorough the common carotid artery and guided to the periinfarct brain region with an external magnet. Nanocapsules were tracked by MRI and brain delivery was confirmed by post-mortem Prussian Blue staining.

Results: *In vitro*, EPCs secretome induced a significant increase in proliferation of endothelial cells and differentiated OPCs ($p<0.05$, respectively) while it triggered a profound change in NSCs phenotype by showing neuron-like morphology and neurogenesis markers (DCX+ and NeuN+). Secretome was successfully encapsulated into magnetized PLGA nanocapsules which were systemically administered without signs of major adverse effects (no weight loss, no pancreatic/renal/liver toxicity), and were successfully directed to the ischemic brain region by applying an external magnet, preferentially accumulating in the blood vessels of periinfarct areas of the ipsilateral hemisphere.

Conclusions: We have demonstrated that EPCs secretome promotes angio-neurogenic processes *in vitro* and that this product could be loaded into PLGA nanoformulations for its safe and targeted delivery into ischemic brain areas to enhance tissue repair.

The role of posttranslational modifications of amyloid peptides in Alzheimer's Disease and related neurodegenerative disorders

The Role of Posttranslational Modifications of Amyloid-ß for Neurotoxicity and Resulting Therapeutic Opportunities

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Alzheimer"s disease (AD) and Parkinson disease (PD) are the two most common age-related neurodegenerative diseases. Both disorders characterized by misfolded proteins which form insoluble deposits in human brain. Since their discovery they were believed the reason for the progression of the disorders. In the last 20 years this view has changed.

First there is increasing evidence that soluble oligomeric forms of the later deposited peptides, Abeta, Tau and alpha-Synuclein exhibit there neurotoxicity before they form fibrils and deposits.

Second many posttranslational modifications of these peptides have shown to be faster aggregating into oligomeric forms serving as seeds for aggregation of unmodified peptides.

Accordingly, therapeutic approaches have been derived to reduce their neurotoxic capacity.

We have developed inhibitors of Glutaminyl Cyclase to prevent the N-terminal pGlu-formation of Abeta molecules and therefore reducing their oligomeric and seeding capacity. This project has reached clinical phase 2.

Similar the reduction of already existing pGlu-Abeta molecules and spontaneously formed isoAspartate containing modified Abeta peptides using monoclonal antibodies have reached preclinical stage and demonstrated proof of concept in different mouse models.

Finally, we have recently demonstrated that Abeta molecules are able to cross-seed and accelerate the aggregation of alpha-Synuclein, opening another therapeutic avenue to treat Parkinson"s disease.

Focused ultrasound improves the efficacy of passive immunotherapy targeting pyroglutamate-3 Aβ

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Pyroglutamate-3 amyloid-β (pGlu3 Aβ) is a pathological, highly neurotoxic form of amyloid-β detected in deposits and water-soluble aggregates of human AD brain. Previously, we demonstrated that passive immunotherapy with an anti-pyroGlu3 Aβ antibody lowered plaques and lessened cognitive decline in an AD-like mouse model. Here, we used focus ultrasound (FUS), with intravenous injection of microbubbles, to transiently and focally open the blood brain barrier (BBB) in order to enhance the intravenous delivery of an anti-pGlu3 Aβ monoclonal antibody (mAb). APPswe/PS1dE9 mice (16 mo) were treated with FUS alone, 500 µg murine anti-pyroGlu3 Aβ IgG2a mAb alone (07/2a; a gift from Probiodrug AG, Halle, Germany), a combination of FUS + 07/2a mAb, or PBS. Antibody was injected intravenously just prior to FUS treatment. WT littermates were treated with PBS or FUS. FUS was applied under anesthesia using an 837 kHz transducer (diameter 10 cm, focal length 8 cm) in conjunction with intravenous 100 µl/kg Optison microbubbles. Burst sonications (10ms at 2 Hz) were applied for 100 s at two locations in each hemisphere in the hippocampus. Animals received three weekly treatments. Behavior tests were conducted 1 week later followed by euthanasia. Brains were examined for amyloid burden, inflammation, and microhemmorhage. Combination treatment (mAb+FUS) showed a significant improvement, and 07/2a mAb alone a strong trend, in learning in the Water T-Maze test compared to PBS controls. FUS alone improved learning in WT mice and memory in AD mice in the contextual fear conditioning test compared to PBS controls. The combination treatment significantly lowered the Aβ42 and pGlu3-Aβ plaque load and increased synaptic markers in the hippocampus of AD mice compared to PBS control AD mice. Iba-1-positive, plaque-associated microglia/macrophages were observed in AD mice with Ab alone and mAb+FUS, while mAb+FUS also induced Ly6G+ monocyte/granulocyte/neutrophil infiltration and association with Aβ plaques. Importantly, microhemorrhage were not increased following FUS, mAb or the combination treatment. Our results suggest that FUS may be a useful tool for facilitating the efficacy of anti-pyroGlu3 Aβ mAb immunotherapy presumably by enhancing delivery to the brain, resulting in better Aβ clearance, synaptic protection and hippocampal function. Interestingly, the combination treatment resulted in the presence of peripheral immune cells within plaques. Thus, FUS may have therapeutic potential when used in combination with anti-pyroGlu3 Aβ mAbs for AD treatment.

Characterization of PBD-C06 - an anti-pyroglutamate-3 Aβ antibody for immunotherapy of Alzheimers Disease

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Pyroglutamate (pGlu)-Aβ is an N-terminally truncated and post-translationally modified Aβ epitope and considered a promising target for immunotherapy against Alzheimer"s Disease (AD). Antibodies against this neoepitope have the potential to clear highly neuro/synaptotoxic forms of soluble and aggregated Aβ peptides, which are implicated with cognitive decline in AD patients. Here, we describe a novel humanized IgG1 antibody, PBD-C06, that specifically targets pGlu3-Aβ-containing neurotoxic peptides and aggregates. PBD-C06 is a single epitope, N-terminal-specific antibody, which binds with high specificity to pGlu3-Aβ monomers, oligomers and fibrils, including mixed aggregates of unmodified Aβ and pGlu3-Aβ peptides. The murine precursor antibody has been described to reduce soluble and insoluble Aβ and to delay cognitive decline in several murine AD-models. For therapeutic development, a sequence optimization process of the precursor antibody included humanization by CDR-grafting and 3D-structural analysis of

PBD-C06:pGlu3-Aβ complexes. Thereby a framework residue was identified that is critically involved in target binding and was exchanged to increase binding affinity. In order to adress safety concerns typically seen with many anti-Aβ antibodies, immunogenic epitopes within the PBD-C06 sequence were exchanged to prevent the generation of anti-drug antibodies in humans. Furthermore, PBD-C06 was modified in the Fc-region to avoid complement-mediated inflammatory responses, with the aim to lower the risk for ARIA-E and thereby achieving a favorable safety profile in the clinic. PBD-C06 is thus an optimized, anti-pGlu3-Aβ clinical candidate antibody ready for entering pre-clinical development and IND-enabling studies.

Differential aggregation and deposition of post-translationally modified Aβ peptides in Alzheimer"s disease

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Objective: Aβ derives from the amyloid precursor protein (APP) during sequential proteolytic processing by β- and γ-secretase. Some mutations associated with familial AD (FAD) are found within the Aβ domain and affect the conformation and aggregation of the peptide. However, such mutations are rare and account for only a very small number of cases. Aβ can undergo several distinct post-translational modifications, including formation of pyroglutamated and phosphorylated species. We recently demonstrated that Aβ undergoes phosphorylation by distinct protein kinases that affect its aggregation and toxic properties. We have now analyzed the deposition of different modified Aβ variants in human brain, and other species with similar Aβ amino acid sequence, including nonhuman primates and canines.

Methods: We applied cell biological, biochemical, biophysical and neuropathological methods to characterize the phosphorylation of Aβ in cell culture models as well as in brains of human AD cases and transgenic mice.

Results: Phosphorylated Aβ shows increased propensity to form oligomeric and fibrillar aggregates and adopt β-sheet conformation, depending on the site of phosphorylation. By using highly specific phosphorylation state specific antibodies, we demonstrate abundant presence of phosphorylated Aβ in brains of human AD cases, APP transgenic mice, canines and nonhuman primates. Phosphorylated Aβ species were detected in extracellular plaques, inside of neurons, and in cerebral vessels, thereby indicating a contribution to all characteristic Aβ associated lesions in AD brains. Phosphorylated Aβ species appear enriched in clinically manifested AD as compared to pathologically preclinical stages. Combined analysis of pyroglutamated and phosphorylated Aβ species in different lesions allowed the distinction of pathological subgroups classification β-amyloidosis.

Conclusions: Phosphorylated Aβ peptides are common and abundant species in human AD brains, transgenic mouse models, and species with the identical amino acid sequence to humans. These variants could be further explored as targets for AD therapy and prevention as well as markers for differential diagnosis.

Monoclonal antibodies targeting isoaspartate-modified amyloid peptides

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Objective: The formation of b-amyloid (Ab) peptides is causally involved in the development of Alzheimer"s disease (AD). As a result of peptide ageing and activation of modifying enzymes, a significant proportion of Ab is posttranslationally modified, which might induce toxicity. Therefore, modified Aβ peptides represent interesting

anchor points for passive immunization. We here present monoclonal antibodies directed against isoaspartate (isoAsp)-modified Aβ and their successful application in 5xFAD transgenic mice.

Methods: The characterization of the antibodies was done using surface plasmon resonance and isothermal titration calorimetry. In a first approach, 5xFAD mice were treated by weekly intraperitoneal injection of two doses of isoAsp antibody (150 and 500 µg per week) or isotype control antibody (IgG2a, 500 µg). The treatment outcome was evaluated using immunohistochemical and ELISA methods. In a further study, 5xFAD mice (n=9-12 per group) were treated by weekly intraperitoneal injection of isoAsp antibody (300 µg per week), isotype control antibody (IgG2a, 300 µg) or antibody 3D6 (IgG2a, 300 µg). The treatment was initiated at three months of age and pursued for nine months in total. The treatment outcome was evaluated using immunochemical methods and by assessing behavior (at 11months of age) in elevated plus maze (EPM), pole test, contextual fear conditioning (CFC) and Morris water maze test paradigms (MWM).

Results: We selected a monoclonal antibody showing a KD value of 6 nM for isoAsp7-Aβ and displaying ~ 400fold selectivity over non-modified Aβ. The modified peptide was observed in brain from transgenic mice. Based on Aβ extraction and ELISA quantification, we conclude that the isoAsp content is low in mice with onset of pathology (4%) and increases with ageing of 5xFAD mice. Treatment of transgenic mice (beginning at 3 months of age) led to a reduction of isoAsp-modified and total Aβ load, suggesting that selective opsonization of modified amyloid is eliciting phagocytosis of non-modified peptides. In the second trial, we observed a significantly lower time spent in open arms in EPM associated with isoAsp- and 3D6 treatments. In CFC, we detected a significant difference in context memory between WT and isotype control treated mice. The isoAsp- and 3D6 antibody treated groups did not significantly differ to WT and isotype control. Finally, also in Morris water Maze only isoAsp-antibody treated animals were not significantly different to WT animals after 4 days of training.

Conclusion: The results support the general concept that modified Aβ peptides, which might be underrepresented compared to full-length Aβ, are attractive targets for immunotherapy. The attractiveness is due to a relatively low concentration of modified Aβ in brain and absence in the periphery. The results merit further investigation of isoAsp-modified antibodies as potential protein drugs.

Targeting acute pathomechanisms following subarachnoid hemorrhage

Micro-ischemia – a novel mechanism of brain damage after subarachnoid hemorrhage

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Subarachnoid hemorrhage (SAH) is a subtype of hemorrhagic stroke with a particularly high mortality and morbidity which affects relatively young patients. The pathophysiology of SAH can be divided into three temporally distinct phases which are all characterized by cerebral ischemia. While the reasons for cerebral ischemia in the hyper-acute and in the late phase after SAH are well known, i.e. intracranial hypertension and delayed vasospasm, respectively, the occurrence of cerebral ischemia between the first hour and the fifth day after hemorrhage, i.e. the period of time with the highest mortality after SAH, remains largely unknown.

The current talk will focus on the effects of SAH on the morphology and functionality of pial and intraparenchymal cerebral vessels. By using in vivo models of SAH in mice in combination with two-photon microscopy we characterized microvascular spasm and evaluated their effect on cerebral perfusion. In additional experiments we evaluated the effect of subarachnoid blood on neurovascular reactivity and coupling. Both sets of experiments demonstrate that ischemia after SAH is caused by pial microvasospasms and subsequent microthrombosis in combination with a complete loss or even an inversion of neuro-vascular coupling. Hence, any regional neuronal activity results in regional mismatches of cerebral blood flow and metabolism thereby inducing ischemia on the cellular level ("micro-ischemia").

Spreading depolarization and inverse neurovascular coupling after subarachnoid hemorrhage

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The term neurovascular coupling describes increased regional cerebral blood flow (rCBF) in response to physiological neuronal activation and decreased rCBF in response to neuronal deactivation. Similarly, rCBF can increase in response to pathological forms of neuronal activation. Those are characterized by an abrupt onset followed by a plateau phase of sustained neuronal depolarization. Two fundamental spectra of sustained depolarizations have been described: (1) the spectrum of epileptic seizures, which despite their dramatic associated changes in neural firing are characterized by relatively mild sustained depolarization; and (2) the spectrum of spreading depolarizations (SD) associated with near-complete sustained depolarization. SD moreover displays abrupt, near-complete breakdown of the transmembrane ion gradients, neuronal edema, mitochondrial depolarization, glutamate excitotoxicity, loss of neuronal firing (=depression) and propagation in the tissue. The SD continuum describes the spectrum from short-lasting SDs in metabolically intact tissue to SDs of intermediate duration to terminal SD in severely ischemic tissue.[1] The term "SD continuum" highlights that there are overlaps but also large variations in mechanistic aspects along the continuum determined by the local tissue conditions. This also applies to the neurovascular responses to SD. Thus, SD induces either transient hyperperfusion in normal tissue (= normal neurovascular response); or severe hypoperfusion (= inverse neurovascular response) in tissue at risk for progressive injury. This hypoperfusion, termed "spreading ischemia", runs together with the SD in the tissue, delays its recovery and increases the risk for cell death.

The inverse neurovascular response to SD was originally discovered in a rodent model mimicking the conditions present following aneurysmal subarachnoid hemorrhage (aSAH).[2,3] In patients with aSAH, this was found a decade later.[4] Interestingly, aSAH patients could also have inverse neurovascular responses to electrographic seizures.[5] In a complimentary fashion, it was then found in brain slices and animals that inverse neurovascular responses also occur in response to physiological neuronal activation after SAH.[6,7] Inverse neurovascular responses may thus occur on all levels from physiological to increasingly pathological types of neuronal activation after aSAH. Among these, the inverse neurovascular response to SD is of outstanding clinical importance because this may directly initiate infarction.[8]

References

1. Dreier, J.P. *Nat. Med* **17**, 439-447 (2011).
2. Dreier, J.P.*, et al. J Cereb Blood Flow Metab* **18**, 978-990 (1998).
3. Dreier, J.P.*, et al. J Physiol* **531**, 515-526 (2001).
4. Dreier, J.P.*, et al. Brain* **132**, 1866-1881 (2009).
5. Winkler, M.K.*, et al. Epilepsia* **53 Suppl 6**, 22-30 (2012).
6. Koide, M.*, et al. Proc Natl Acad Sci U S A* **109**, E1387-1395 (2012).
7. Balbi, M.*, et al. J Cereb Blood Flow Metab* **37**, 3625-3634 (2017).
8. Luckl, J.*, et al. Brain* **141**, 1734-1752 (2018).

Blood-brain barrier and white matter changes after subarachnoid hemorrhage: injury and repair

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Most research on subarachnoid hemorrhage (SAH) has focused on changes in cerebral blood flow and mechanisms of grey matter injury. There is much less information on blood-brain barrier (BBB) and white matter (WM) changes. The aim of this presentation is to (1)review the BBB and WM changes that occur in animal models of SAH as well as patients; (2) describe how those changes may impact overall SAH injury and recovery; (3) provide evidence on potential mechanisms underlying SAH-induced BBB and WM injury. Mechanistically, there will be a focus on the role of global cerebral ischemia at SAH ictus, the importance of clot-derived factors and the significance of hemorrhage

extension into the ventricular system in inducing injury and hydrocephalus. SAH-induced hydrocephalus is a known risk factor for poor outcome.

TOPSAT2 - A trial for poor grade subarachnoid haemorrhage patients

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This RCT compares the efficacy of a strategy of early aneurysm treatment in a population of WFNS grade 4-5 (poor grade) aneurysmal subarachnoid haemorrhage (aSAH) patients in comparison with a strategy of treatment of aneurysm after neurological improvement (to WFNS grade 1-3).

This is a prospective, randomised, parallel group study with blinded outcome evaluation comparing two management strategies. Primary outcome is functional outcome at 12 months determined by ordinal analysis of modified Rankin score (mRS).

346 patients aged 18-80 years old and admitted to neuro ITU with WFNS grade 4 or 5 aSAH will be recruited in UK and Europe.

Patients will be randomised to early treatment (within 72 h of ictus) or treatment after neurological recovery using a web-based randomisation service. Outcome questionnaires will be sent to patients at 6 and 12 months.

Site and patient recruitment is ongoing.

There is evidence of substantial variation in practice for patients with poor grade SAH. This trial will demonstrate whether early aneurysm treatment achieves a better outcome on average.

Targeting the interplay between stem cells and neuroinflammation in stroke regeneration

Enhancing stem and immune cell-mediated regeneration after stroke

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Ischemic stroke triggers inflammation, activation of microglia, as well as infiltration of immune cells from the blood, including monocytes. Recent studies indicate that there is a cross-talk between immune cells and brain"s own endogenous as well as grafted neural stem/progenitor cells (NSPCs). Activated microglia and infiltrating immune cells produce pro- and anti-inflammatory mediators which could be detrimental and/or beneficial, respectively, for cell genesis from endogenous and grafted NSPCs. Taken together, these observations point to two cell-based therapeutic strategies which potentially could promote functional restoration after stroke: (1) to deliver or activate endogenous NSPCs for modulation of inflammation, and at a later stage, replacement of those cells which have died; (2) to increase number of immune cells beneficial for cell genesis and other regenerative responses.

We demonstrated that endogenous monocytes home to the stroke-injured hemisphere and that the monocyte-derived macrophages (MDMs) exhibit a high degree of functional plasticity, changing from pro- to anti-inflammatory phenotype during the first weeks following the insult. Blocking monocyte recruitment during the first week after stroke abolished long-term behavioral recovery. We then demonstrated that MDMs, primed in vitro to become anti-inflammatory and administered into cerebrospinal fluid of stroke-subjected mice, infiltrate the ischemic hemisphere and promote recovery of motor and cognitive functions.

Ischemic stroke, leads to formation of new striatal neurons from NSPCs in the subventricular zone (SVZ) of adult rodents. Concomitantly with this neurogenic response, SVZ exhibits activation of resident microglia and infiltrating monocytes. We showed that depletion of circulating monocytes during the first week after stroke, enhances striatal neurogenesis, most likely by increasing short-term survival of the newly formed neuroblasts in the SVZ and adjacent striatum. Blocking monocyte recruitment did not alter the volume of the ischemic lesion but gave rise to reduced astrocyte activation in SVZ and adjacent striatum, which could contribute to the improved neuroblast survival. A similar decrease of astrocyte activation was found in and around human induced pluripotent stem cell (iPSC)-derived NSPCs transplanted into striatum at one week after stroke in monocyte-depleted mice. However, there was no effect on neurogenesis in the graft as determined 8 weeks after implantation. Our findings demonstrated that a specific cellular component of the early inflammatory reaction in SVZ and adjacent striatum following stroke, i.e., infiltrating monocytes, compromises the short-term neurogenic response neurogenesis from endogenous NSPCs. We provide strong evidence that spontaneously recruited monocytes to the injured brain early after the insult contribute to long-term functional recovery after stroke most likely through affecting neuronal plasticity.

Adjuvant stroke treatment - do we need stem cells for cellular regeneration?

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Stem cells from different origins have been shown to induce neuroprotection and neuroregeneration after induction of cerebral ischemia. Yet, grafted cells mediate these aforementioned effects by indirect mechanisms, among which is the secretion of extracellular vesicles (EVs). Indeed, the application of both stem cells and stem cell derived EVs appears to be equally effective against experimental stroke. Although EV application appears to be safe, circumventing possible stem cell associated side effects, long-term data on EV application does not exist. Rather, boosting post-stroke endogenous neuroregenerative mechanisms such as stem cell recruitment and others may appear to be more attractive and safer in the long run. In this context, the application of remote ischemic post-conditioning (rPostC), which is defined as a transient and subcritical period of peripheral organ ischemia following a stimulus of injurious cerebral ischemia, has recently gained increasing interest in experimental stroke research. We have previously shown that very late but not acute induction of hind limb rPostC yields sustained neurological recovery in mice via a reversal of post-stroke immunosuppression. Further underlying mechanisms, however, have not been addressed at that time. Herein, we demonstrate that timing of induction of rPostC differentially affects resistance of bone marrow (BM) derived mesenchymal stem cells (MSCs) against in vitro hypoxia. Moreover, secretion patterns of MSC-derived extracellular vesicles (EVs), which are regarded to play a pivotal role in mediating MSC-induced neuroprotective effects, differ significantly depending on the experimental paradigm of rPostC. As such, very late rPostC generates increased numbers of MSC-derived EVs that display enriched concentrations of selected miRNAs and proteins, which in turn have been proven to yield cell survival of neurons exposed to ischemic injury. Indeed, the infusion of EVs derived from MSCs that were obtained from mice exposed to very late but not acute rPostC shows a higher therapeutic potential against middle cerebral artery occlusion, indicated by a better behavioral test performance in comparison to EVs derived from native MSCs or mice treated with PBS only. In conclusion, very late rPostC mediates its neuroprotective effects by modulating EV secretion patterns of endogenous BM derived MSCs, thus contributing to the reduction of histological brain injury, stimulated neuroregeneration, and increased neurological recovery.

The dynamic pattern of microglia polarisation and their multidimensional effects on neurorepair by affecting endogenous neural stem cells *in vitro* and *in vivo*

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Microglia are activated after lesions to the brain and polarize towards a classic “M1” pro-inflammatory or an alternative “M2” anti-inflammatory phenotype following typical temporospatial patterns, contributing either to

secondary tissue damage or to regenerative responses. They closely interact with endogenous neural stems cells (NSC) residing in distinct niches of the adult brain. The current study aimed at elucidating the plasticity of microglia polarization and their differential impact on NSC survial, proliferation, migration and differentiation potential. Primary rat microglia in vitro were polarized towards an M1 phenotype by LPS, or to an M2 phenotype by IL4, while simultaneous exposure to LPS plus IL4 resulted in a hybrid phenotype expressing both M1- and M2-characteristic markers. M2 microglia were less motile but exerted higher phagocytic activity than M1 microglia. Defined mediators switched microglia from one polarization state to the other, a process more effective when transforming M2 microglia towards M1 than vice-versa. Polarized microglia had differential effects on the differentiation potential of NSC in vitro and in vivo, with M1 microglia promoting the generation of astrocytes, while M2 microglia supported neurogenesis. Regardless of their polarization, microglia inhibited NSC proliferation, increased NSC migration, and accelerated NSC differentiation upon mitogen withdrawal. Overall, this study reveal the complex conditions governing the dynamics of microglia polarization, and the multifaceted effects of differentially polarized microglia on key functions of NSC in vitro and in vivo. Data will inform the prospective development of innovative therapeutic concepts supporting the regenerative capacity of the brain, e.g. after cerebral ischemia.

Translational stem cell research for brain repair: where do we stand and where do we go?

Cell therapies for stroke with iPS cell and reprogramming technologies

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In recent years, the recovery phase of stroke attracted much of the attention of researchers and clinicians, and currently is considered as most suitable target for the stroke therapy. This is justified by the long-term therapeutic window and also intrinsic plasticity-based mechanism of recovery which is operating in the brain and represents suitable target of the therapy. Cells from different sources have been tested for their ability to reconstruct the forebrain and improve function after transplantation in animals subjected to stroke.

We have recently shown improved functional recovery after transplantation of human reprogrammed induced pluripotent stem cells (iPSC)-derived cortical neuronal precursors in a rat model of cortical stroke.In our recent study, we used a rabies virus (RV)-based strategy to explore whether host cells can establish functional synaptic connections with transplanted cells. Two or five months after transplantation of modified It-NES cells in a rat stroke model, we injected the RV in the location of the graft. Expression of TVA receptor in the mature neurons (synapsin I+) generated from grafted cells makes them suitable for infection with the RV. The presence of Rabies-G glycoprotein in these cells allows the virus to infect the cells that connect to them by functional synapses. Therefore, grafted and infected cells will express nuclear GFP and cytoplasmic RFP while the ones connected to them will only present RFP in the cytoplasm.

Immunohistochemical analysis of injured and transplanted brains one week after the infection with RV revealed the presence of RFP+ neurons in different areas, some of them located far away from the implantation site. Using electrophysiological recordings in vivo and optogenetics in brain slices recordings combined with patch-clamp we demonstrated, for the first time, that intracortical grafts of human iPSC-derived cortical neurons establish functional afferent synaptic connections with stroke-injured brain and respond to peripheral sensory stimulation. We currently study how the reconstruction of stroke-lesioned network by grafted stem cell-derived neurons contribute to post-stroke recovery.

Recent papers demonstrated rapid and efficient conversion of human somatic cell to mature neurons by overexpressing transcription factor combinations. We have attempted to generate projection cortical neurons by direct reprograming of somatic cells. We demonstrated that a combination of three transcription factors convert human fibroblasts to functional excitatory cortical neurons. Single-cell analysis revealed a complex gene expression profile, a subpopulation of neurons displaying a molecular signature similar to human fetal primary cortical neurons.

Our findings indicate that functional excitatory cortical neurons, generated by reprogramming of human somatic cells is feasible and could be further developed for cell therapy strategies.

Bio-engineering strategies with stem cells for chronic stroke

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The clinical translation of cell therapy for stroke is rapidly progressing. However, several key considerations for delivery remain poorly addressed. Notably, the biophysical characterization of cell suspensions (e.g. cell density), as well the exposure of biomechanical effects (e.g. sedimentation, exposure to shear stress) during the delivery process are key components to ensure that a therapeutic effect is guaranteed. These bioengineering strategies ensure quality control of cell therapy, but also provide the basis to further improve the delivery process and afford an efficient therapeutic response. Additional bioengineering strategies, such as delivery of cells in hydrogel, to ensure improved survival or dispersion at the site of implantation, and potentially tissue reconstruction, are also considered. As cell therapy moves from a proof-of-concept therapeutic to a viable clinical therapy, added value will be given to these new treatments by considering additional engineering strategies.

Never too late! Extending and improving the time window for stroke

Time windows for thrombolysis and thrombectomy

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After the successful NINDS rt-PA for Acute Stroke Study in 1995, thrombolysis with intravenous alteplase was limited to 3 hours following known stroke onset. Later, after ECASS II and ECASS III, some began using a time window of 4.5 hours in certain circumstances. The key to these time windows was the determination of the stroke onset time. In the case of unwitnessed onset, clinicians began to substitute the time the patient was last known to be well, or LKW. Underlying all of this emphasis on clocks were two key assumptions. First—and quite well supported by empiric data—was the observation that the odds of a favorable outcome declined with time. This observation implied, as the saying goes, time is brain, and minutes matter. At some point in time the odds of a favorable outcome reached 1.0, implying futility in offering treatment beyond that time window. The second assumption was that time measured with a clock correlated well with progression of tissue irreversibility. This assumption, while reasonable, was supported by far less empiric data. In fact, case series and anecdotal observations raised considerable doubt that the clock provided a suitable indicator of tissue irreversibility: many patients treated within the time window failed to improve; sometimes patients treated beyond the time window did improve. In parallel with the development of stent-retriever endovascular thrombectomy catheters, imaging technology improved sufficient to allow rapid determination of tissue salvagability. After several iterations, it has become clear that poorly perfused tissue exhibiting few correlates of cell death (e.g. diffusion restriction) remains potentially salvageable. Very new data confirms that reperfusion—whether by thrombectomy or thrombolysis—may be associated with favorable outcome even as late as 24 hours after LKW, assuming the imaging detects patterns consistent with reversible injury. As important as the clock was in the early days, just so we now depend on imaging to select patients for recanalization. Imaging allows a patient-centric approach, within limits, such that modern time windows for thrombolysis and thrombectomy reflect a combination of clock time and imaging signature.

The longest window: cell therapies to rebuild brain

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Effective and safe treatment with tissue plasminogen activator (tPA) remains at 4.5 hours after stroke onset. Stem cell-based regenerative medicine has extended this tPA-neuroprotective therapeutic window well beyond the critical hours during the ischemic injury to days, weeks, months and even years after the stroke episode. Rebuilding the stroke brain is not amenable to any rogue attempt to market stem cell therapy as a magic bullet. A general understanding of the cell dose, timing and delivery is recognized, and a wealth of knowledge has revealed the mechanisms of action underlying stem cell therapy for stroke. Limited clinical trials are underway, with consistent reports demonstrating the transplanted cells' safety profile, while the full efficacy readouts are forthcoming. The optimal transplant regimen may still require tailoring to specific acute and chronic stroke conditions. To this end, a careful assessment of the disease pathology and the treatment continuum may reveal that combination of neuroprotection in the acute period and regeneration in post-acute to chronic stages of stroke, as opposed to solely pursuing stand-alone treatments, will improve stroke outcomes. A cascade of cell death events from stroke onset and progression necessitates multiple neuroprotective and regenerative approaches. Harnessing the brain microenvironment prior to or early on after injury to actively respond to the subsequent transplantation of healthy stem cells and their therapeutic trophic factors and exosomes, and viable mitochondria may prove that the longest window to rebuild the brain is mutually linked to the initial acute investment of protecting the brain from primary and secondary cell death processes associated with stroke. The gaps between short and long therapeutic windows of stroke can be bridged by combined neuroprotective and neuroregenerative treatments.

Long-term neuroinflammation during functional recovery from stroke

State-of-the-art: what do we know about long-term neuroinflammation during stroke recovery?

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Functional deterioration after stroke is poorly understood and poorly treated despite its critical relevance for the quality of life of stroke survivors. Several studies highlight that complex immune responses take place beyond the acute phase of stroke that might participate in long-term secondary complications. Inflammation has been regarded as a target for pharmacological intervention to reduce brain damage in acute stroke. Nevertheless, certain inflammatory responses are necessary to promote repair processes because mechanisms involved in resolution of inflammation are able to promote functional recovery. This concept suggests that the initial inflammatory reaction triggers a set of responses that may improve the functional outcome in the long term. Several strategies designed to dampen innate immune responses, comprising the activation of innate immune cells and complement pathways, effectively reduce acute brain damage in experimental animal model of stroke. Some long-term studies show that these benefits are persistent, in spite that certain inflammatory molecules, e.g. toll-like receptors, IFNg, complement proteins, are important in neurogenesis and regenerative processes. Therefore, there seems to be a time-window for acting on acute inflammatory responses, and intervention on certain specific pathways may be superior to global reduction of inflammation. Different immune cells are attracted to the injured brain tissue. Monocytes reach the brain within the first 4 days post-stroke, acquire features of tissue macrophages, and persist for long in the damaged tissue. While some studies support that macrophages play detrimental functions, emerging evidence suggests that they play critical roles in long-term functional recovery. Lymphocytes seem to exert deleterious functions in acute stroke by promoting thromboinflammation through mechanisms that do not involve adaptive immune responses. However, lymphocytes sense signals of brain damage from the periphery, they reach the brain in higher numbers in chronic phases, and several lines of evidence support that adaptive immune responses may play a role in functional impairment. More efforts are needed to understand the role of chronic inflammatory and immune responses in stroke patients because targeting these responses beyond the acute phase may still improve life after stroke.

MiRNA therapy improves long term affective dysfunction due to ischemic stroke

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Post-stroke depression, a long-term consequences of ischemic stroke, occurs more frequently in older women and can reduce the quality of life, hamper stroke recovery and increase mortality. Conventional anti-depressant therapies are not as effective in this population of PSD patients. Using an animal model, we tested the hypothesis that a neuroprotectant would be effective for alleviating PSD by reducing striatal infarct volume and preserving mesostriatal reward pathways that are implicated in depression. Previous work from our lab has shown that microRNA (miR)363-3p reduces infarct volume in acyclic middle-aged female during the acute phase (5d) of stroke. Using this model, sham and stroke animals were assigned to either mir363-3p treatment or scrambled oligos (control) and followed for 100+ days after stroke. T Maze cost/benefit task (TMCBT), Social Interaction (SI) and Forced Swim Test (FST) were used to assess depressive-like behaviors. Thereafter, rats were injected with Fluorogold (Flg) into the left and right striatum and to assess the mesostriatal projection pathway. Our results show that after stroke, the control group (MCAo+scrambled) displayed reduced high-reward choice (anhedonia) at 98d in the TMCBT. Similarly, at 100d, social interaction decreased 3-fold from the baseline, and immobility (helplessness) was significantly increased as measured by the FST. In contrast, animals treated with mir363-3p did not show reduced social interaction or immobility and were significantly higher than controls in high-reward choice test. Cytokines IL-6 and TNF-alpha lelves were transiently elevated in the control group and at 3+ months after stroke circulating BDNF were lower in the scrambled group as compared to Mir363-3p group. This decrease in BNDF was also accompanied by a reduction in the number of retrogradely-labeled cells in the SNc and VTA in scrambled group. These data are consistent our hypothesis that mir363-3p treatment after stroke will preserve the meso-striatal "reward" pathway in the ischemic hemisphere and ameliorate chronic development of PSD.

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Long-term T cell responses in the ischemic brain

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Introduction: T cells exhibit both protective and detrimental effects in the first days following stroke, depending on several factors including subtype, location, and timing of egress. But T cell egress into the post-stroke brain might actually peak weeks after stroke onset, suggesting an additional long-term role for the adaptive immune system in the recovering CNS.

Methods: To determine the specific role of long-term CD8 T cell egress, male C57BL/6 mice (Jackson Labs, 8-14 weeks) were subjected to 60-min transient middle cerebral artery occlusion (tMCAo) with up to 30 days of recovery. Immune subpopulations were quantified using histology and flow cytometry, infarct volume by MRI, and functional recovery by rotarod behavioral test. Mice were randomized to continuous CD8 T cell depletion using CD8-specific antibody (2.43 clone ip) or isotype control antibody cohorts starting 10 days after tMCAo to test the relevance of delayed T cell egress on functional recovery.

Results: Post-stroke mice have a delayed influx of leukocytes, with higher numbers of CD4 and CD8 T cell numbers in the ischemic hemisphere at 14 and 30 days after stroke. Moreover, T cell numbers correlated with the functional recovery, as mice with higher CD4 and CD8 T cells in the ipsilesional hemisphere exhibited worse motor deficits. Antibody-mediated depletion reduced CD8 T cell numbers in the ischemic brain at 30 days post-tMCAo. CD8 T cell-depleted mice had 2.5-fold reduced leukocyte infiltration in the ipsilesional hemisphere, including reduced cell numbers of macrophages, neutrophils, and CD4 T cells. Also, these mice exhibited a higher percentage of circulating CD4 T cells without significant differences between immune cell numbers in spleen between cohorts.

Conclusion: Our data show that there are long-term CD8 T cell responses in the ischemic hemisphere post-stroke, and that these chronic phase T cells affect general long-term neuroinflammation in the ipsilesional cortex and functional recovery post-stroke, which suggest a potential immunotherapeutic target admissible for weeks after stroke onset.

B lymphocytes cause cognitive decline after stroke

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Stroke increases the risk of subsequent incident dementia. Dr Buckwalter will describe a model of post-stroke cognitive impairment in mice. Mice have normal cognition and hippocampal electrophysiology (long-term potentiation) one week after a cortical stroke adjacent to the hippocampus. Within seven weeks, cognitive impairment and progressive loss of long-term potentiation begin, in parallel with the accumulation of antibody-producing B-lymphocytes in the stroke core, and antibodies in the core and surrounding brain parenchyma. Delayed cognitive impairment can be prevented in this model by ablating B-lymphocytes using both genetic and treatment models. In addition, she will discuss the evidence for B lymphocyte involvement in this process in humans.

Systemic measures of inflammation predict long-term recovery after intracerebral hemorrhage.

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Experimental models offer great tractability to manipulate pathways and improve outcomes after intracerebral hemorrhage. Mechanisms can easily be verified on postmortem brains at multiple time points. However, translational success is limited by the lack of access to patient brains in real time, and thus there is necessary reliance on noninvasive measurements either through neuroimaging or peripheral blood. The data presented will specifically address this translational gap. Measurements of inflammation in the peripheral blood and the associations with long-term outcomes in both murine models and human patients will be discussed.

Current concepts and future avenues in brain-immune interaction after stroke

Understanding the role of the inflammasome in stroke

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It is now well recognised that inflammation plays a major role in the pathophysiology of stroke and as such offers an attractive therapeutic target. The inflammation that occurs in stroke is referred to as sterile since it occurs in the absence of pathogens. Members of the interleukin-1 (IL-1) family, IL-1alpha and IL-1beta, are the primary inflammatory cytokines associated with sterile inflammatory responses. A large multiprotein complex called the inflammasome regulates the release of IL-1 from activated immune and other cells. Inflammasomes are the focus of much recent research in a number of disease conditions, including stroke and other neurological disorders.

Minutes to hours after after a stroke cell death triggers an inflammatory response through the release of danger signals referred to as damage associated molecular patterns (DAMPs) that stimulate pattern recognition receptors (PRRs) on cells of the innate immune system, especially microglia in the brain. Activation of these PRRs leads to the formation of inflammasomes, which subsequently activate caspase-1, which in turn processes pro-IL-1 allowing release of the mature active cytokine.

There is accumulating evidence that different inflammasomes can be activated after stroke and we previously reported that ischaemic injury is dependent on AIM2 and NLRC4 inflammasomes, as well as the inflammasome component ASC. However, despite several recent reports that the NLRP3 inflammasome contributes to cell death in stroke, our previous and current findings suggest that this is not the case, at least in ischaemic stroke.

Following activation of the inflammasome and release of IL-1 from cells after stroke the site of action of the mature active cytokine is still not well defined. To address this we have used cell-specific deletion of the IL-1 type 1 receptor (IL-1R1) and have identified both brain endothelial and neuronal cells as major targets of IL-1 in mediating ischaemic cell death. Cell-specific targeting of IL-1R1 in the brain could therefore have therapeutic benefits in stroke and other cerebrovascular diseases.

The role of Interleukin-17 in acute stroke

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It is well established that the reperfusion of ischemic brain tissue and the immediate activation of CNS resident cells augment a rapidly evolving inflammatory response that leads to an additional infarct growth and neurological deterioration. Recent lines of evidence directly link local inflammatory and immune reactions with the degree of stroke-associated brain damage. In this context, T cells have been shown to be an essential part for the post-ischemic tissue damage and especially IL-17 secreting γδ T cells have been implicated in the pathogenesis of post-stroke inflammation. It seems that γδ T cells are the main IL-17 producing cells in the first days and that the γδ T cell activation initiates a conserved overwhelming detrimental immune response in the ischemic brain. The rapid activation of γδ T cells suggests that the IL-17 production is initiated in an antigen independent manner through cytokines and damage-associated molecular pattern (DAMPs). Effector mechanism of γδ T cell derived IL-17 include the induction of metalloproteinases, proinflammatory cytokines, and neutrophil attracting chemokines in the ischemic brain. In this part of the minisymposium, we will give an overview on the concepts of γδ T cells and IL-17 in stroke pathophysiology and on their potential importance for human disease conditions.

Microglial and macrophagic functions in post-stroke neuroinflammation

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Inflammation is currently considered a prime target for the development of new stroke therapies. In the acute phase of ischemic stroke, microglia are activated, followed by infiltration of circulating immune cells invading the peri-infarct and infarct core. In combination, resident and infiltrating cells secrete cytokines and orchestrate the inflammatory response. Inflammation can be detrimental and beneficial at particular stages after a stroke. It can contribute to expansion of the infarct but is also responsible for infarct resolution, and influences remodeling and repair. Experimental evidence shows that targeting inflammatory cytokines, such as tumor necrosis factor (TNF), holds promise. However, as TNF possess non-redundant protective and immunoregulatory functions, its neutralization carries a potential for unwanted side effects and clinical translation is therefore challenging. This talk provides an overview of the post-stroke TNF response and discusses pharmacological and cellular interventions targeting TNF inflammation after a stroke, which may be used alone or in combination with recanalization therapies. Identification of next-generation neuroprotective therapies should aim at selectively neutralizing pathogenic TNF immune signaling, enhancing tissue preservation, promoting neurological recovery and leaving normal function intact.

The multiphasic systemic immune response after stroke

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Alteration of the peripheral immune system is a key feature of systemic effects after acute stroke. One prominent phenomenon of post-stroke immunomodulation is an immunosuppressive syndrome in the subacute phase after stroke. However, recent works by us and others have demonstrated a strong over-activation of peripheral immunity in the acute phase and a low-grade chronic inflammatory state in the chronic phase after experimental and clinical stroke. These findings have generated the novel concept of a multiphasic systemic immune reaction to acute stroke. This presentation will give an overview of the experimental data establishing this novel concept. Furthermore, I will particularly focus on the clinical consequences of this dysbalanced peripheral immune homeostasis after stroke. While the acute sterile inflamatory response leads in mice to cytokine-induced sickness behavior, stroke patients suffer from depression-like symptoms which may be exacerbated due to inflammatory mechanisms. Additionally, the pro-inflammatory response after stroke also affects other inflammatory comorbidites prevalent in stroke patients such as atherosclerosis. These experimental findings indicate an important role of peripheral inflammatory mechanisms for comorbidities and outcome of stroke patients.

Neutrophils and stroke

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Inflammation plays an important role in the pathogenesis of ischemic stroke including an acute and prolonged inflammatory process. The role of neutrophil granulocytes as first driver of the immune reaction from the blood site is under debate due to controversial findings.

Using transgenic mice we studied the dynamics of neutrophils and the interplay with microglia after cerebral ischemia using intravital two-photon microscopy. We demonstrate the infiltration of neutrophils into the brain parenchyma and confirm a long-lasting contact between neutrophils and microglia as well as an uptake of neutrophils by microglia clearing the brain from peripheral immune cells.

Effect of Sex and Age on Post-ischemic Outcome and Therapy

Sex differences in response to NKCC1 inhibition following ischemic stroke

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The WNK-SPAK/OSR1 kinases and their substrate Na+-K+-Cl- cotransporter (NKCC1) play a role in cerebral edema and neurological functional deficit after malignant ischemic stroke under normotensive conditions. Inhibition of NKCC1 in the central nervous system is of interest in treatment for ischemic stroke. However, NKCC inhibitor bumetanide (BMT) has poor penetration into the brain. In this study, we investigated the efficacy of two novel NKCC1 inhibitors, a lipophilic BMT prodrug STS5 and a novel NKCC1 inhibitor STS66, on reducing ischemic brain injury. Malignant transient ischemic stroke was induced in normotensive C57BL/6J mice with 50-min occlusion of the middle cerebral artery (t-MCAO) and reperfusion. Mice were randomly assigned to receive vehicle DMSO, BMT, STS5, or STS66 with an initial dose at 3 h post reperfusion or stroke and daily for 1-5 days. BMT, STS5 or STS66 significantly reduced acute infarction and cerebral swelling after t-MCAO in male mice but less profound in female mice. STS66-treated mice showed better improvement of survival and sensorimotor functional recovery during 1-14 days post t-MCAO. AngiotensionII-induced hypertensive male mice displayed significant activation of the WNK-SPAK/OSR1-NKCC1 cascade and worse ischemic brain injury than normotensive male controls after permanent

ischemic stroke (p-MCAO). STS66 and BMT treatment reduced infarction and improved sensorimotor function in male mice with hypertension.

Taken together, the novel NKCC1 inhibitor STS66 is superior to BMT in reducing ischemic infarction, swelling, and neurological deficits in malignant transient ischemic stroke as well as in permanent focal ischemic stroke with hypertension comorbidity.

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Sex and Age Differences in Post-stroke Inflammation and Microglial Activation

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Introduction: Post-stroke inflammation is both causative and resultant to ischemic brain damage. Activation of the resident immune cells, microglia, plays a central role in initiating and perpetuating the immune responses. Our experimental studies have reported that sex and age related differences exist in ischemic stroke outcomes, which mirrors clinical epidemiologic and pathologic data. How sex and age impact on the immune response to stroke is not known. We hypothesized that immune responses and microglial activation after stroke have sex-specific characteristics that changes with age, and plays significant roles in mediating stroke sensitivity.

Methods: Both wild type (WT) and four-core genotype (FCG) mice of different ages were used. Stroke in post-natal day 10 (P10) pups was induced by Rice-Vannucci Model; young adult (8-12 weeks) and aged (18-20 months) mice were subjected to middle cerebral artery occlusion (MCAO) model. Stroke outcomes were measured by morphological changes in the brain and behavior tests 3 days after ischemia. Flow cytometry was performed to analyze immune cell infiltration in the ischemic brain. Microglial activation was determined by expression of both cell membrane and intracellular markers with flow cytometry and immunofluorescence staining. Circulating hormones (estrogen, testosterone) and cytokines (TNF-α, IL-1β) were examined in serum with MultiPlex.

Results: P10 male pups had worse stroke outcomes than females, and this male sensitive ischemia phenotype lasted into the adulthood. Gonadectomy abandoned the sex difference in young mice. In contrast, female aged mice have worse stroke outcomes than males and the female sensitive stroke phenotype was seen in XXF/XXM vs. XYF/XYM mice. More activated microglia were seen in aged vs. young ischemic brains shown by IHC. Flow cytometry showed significantly more immune cells infiltrated in neonatal male vs. female ischemic brains, and males had more MHCII+ microglia after stroke. However, aged female ischemic microglia exhibited higher MHCII expression than male microglia, and more immune cell infiltration was seen in female brains. Serum cytokine levels also showed a similar sex difference pattern. Circulating hormone levels were equivalent between male and female neonates or aged mice.

Conclusion: Sex specific immune responses exist as a "signature" in XX vs. XY mice after ischemia. Hormonal effects direct the sex difference in young adult mice; however, chromosomal effects may mediate stroke sensitivity and sexual dimorphism in immune responses/microglial activation in neonates and the aged.

Sexual dimorphism in inflammasome activation exacerbates ischemic brain damage in reproductively senescent (RS) female rats

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A woman's risk of a stroke increases exponentially following the onset of menopause, and underlying mechanisms remains unknown. The current study tests the hypotheses that: (1) inflammasome activation is significantly higher in the brain of RS females as compared to their young counterparts and senescent male rats, (2) RS triggers an innate immune inflammatory response in the ovaries that spreads to the brain, making the brain more susceptible to ischemic damage. We tested our hypotheses using Sprague–Dawley rats of both sexes (6–7 and 9-12 months). The estrous cycles of female rats were monitored for 14-20 days prior to experimentation by daily examination of vaginal smears. Rats that remain in constant diestrus were considered RS. Rats (n = 4-7) of both sexes and ages were sacrificed and hippocampus, gonads, serum and cerebrospinal fluid (CSF) were collected. Additionally, cerebrospinal fluid (CSF) of women (<40 and >50 age) was obtained. Extracellular vesicles (EV) were isolated from serum and CSF using an Invitrogen kit. Inflammasome proteins caspase-1, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and IL-1β significantly increased in the hippocampus, serum, and ovaries of RSF as compared to YF ($p<0.05$). This was not observed in the hippocampus or gonads of age-matched males. Importantly, EV obtained from RSF contains significantly higher levels of the inflammasome proteins as compared to YF ($p<0.05$). EV containing inflammasome proteins originates in the ovaries of RSF and then are carried to the brain *via* blood. The observed increase in ovary-derived EV containing inflammasome proteins in the brain contributes to the inflammation present in the brain of RSF, and it might exacerbate ischemic brain damage. Future studies investigating the role of ovarian EV in post-ischemic inflammation are underway to understand how modulating EV trafficking can reduce the incidence and impact of cerebral ischemia in post-menopausal women.

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Sex and age differences in the efficacy of miRNA as stroke neuroprotectants

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Short non-coding RNA (microRNA) function as translational repressors and are promising candidates for stroke therapy. However, identifying a suitable miRNA can pose a challenge, especially in populations with comorbid risks. This presentation will discuss our studies of three miRNAs, where the neuroprotective outcomes were influenced by biological sex and age. We used two strategies to identify neuroprotective miRNA: a targeted approach and a discovery approach. In the case of the targeted approach, we focused Let7f, a miRNA that regulates insulin-like growth factor (IGF)-1. Recombinant IGF-1 treatment reduces infarct volume and improves motor behavior in middle-aged females. Our studies showed that antagomirs to Letf7 increased IGF-1 in microglia, and reduced infarct volume and improved sensory motor performance when administered to adult females 4h post stroke. However, this treatment had no effect on adult males, or ovariectomized females, and paradoxically increased stroke impairment in middle-aged females. Our second strategy was based on a discovery approach, where we compared circulating microRNA profiles from groups of adult and middle-aged males and females. Six miRNA, including mir363-3p, were found to be directly correlated with stroke outcomes, where increased expression of these miRNA occurred in groups with the best stroke outcomes. Treatment with mir363-3p mimics improved stroke outcomes in middle-aged females but were surprisingly ineffective in males. Finally, we compared miRNA profiles in astrocytes from adult and

middle-aged male and females. This comparison yielded sex specific regulation of members of the mir17-92 cluster. Mimics of this miRNA improved stroke outcomes in both males and females. Collectively, our studies underscore the hypothesis that stroke therapies are likely to be sex-specific, and drug effectiveness will depend on modifiable and non-modifiable stroke risk factors.

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Role of non-coding RNAs and epigenetics in post-stroke brain is dependent on age and sex

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Stroke is known to alter noncoding RNAs and epigenetic factors. Although rectifying these changes is known to protect the brain, it is essential to identify therapies that are efficacious in both sexes and at different ages. Hence, we tested the therapeutic potential of modulating a microRNA (miR-7), a long noncoding RNA (lncRNA FosDT) and an epigenetic mark (5-hydroxymethylcytosine; 5hmC) as a function of age and sex following transient middle cerebral artery occlusion (MCAO) in rodents. Focal ischemia down-regulated miR-7 in both young/adult and aged rats of both sexes. Replenishing its levels with a miR-7a mimic following transient MCAO significantly decreased the infarct volume and neurological deficits in both sexes and importantly in both young/adult and aged mice. The miR-7a repressed α-synuclein and this effect might be responsible the neuroprotection. LncRNA FosDT is abundantly expressed in the brain and significantly upregulated following transient MCAO. Both male and female FosDT knockout mice showed smaller infarcts and better neurologic outcomes following stroke. FosDT scaffolds the transcription factor REST and its corepressors enabling REST-mediated suppression of neural genes leading to brain damage after stroke. Epigenetic mechanisms contribute to the sex differences in functional outcome after CNS insults. Importantly, 5hmC is considered as a beneficial epigenetic modification that de-repress the genes silenced by DNA methylation. Stroke led to significant increase in the 5hmC levels in mouse brain catalyzed by the enzyme TET3. Inhibition of TET3 exacerbated cortical infarct volume in both male and female mice, and increased the mortality in male mice. The hMeDIP-seq analysis revealed that TET3 knockdown decreases 5hmC peaks in the promoter region of genes known to control oxidative stress and inflammation. TET inducer ascorbate increased cortical 5hmC levels and decreased secondary brain damage in both young and aged, male and female mice after stroke. Inhibition of TET3 prevented ascorbate-induced 5hmC and neuroprotection after stroke indicating ascorbate-induced neuroprotection occurs via TET3. These studies show that epigenetics might useful to develop new therapies to protect brain after stroke. Overall, our results show that modulating certain noncoding RNAs and epigenetics can protect post-stroke brain irrespective of sex and age.

Theranostics in stroke – Novel (dietary) therapeutic and diagnostic approaches in preclinical stroke

Healthy food healthy brain; preventive and therapeutic effect of nutrition

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Abdominal obesity, dyslipidemia, and hypertension, contribute to structural and functional alterations in the peripheral and cerebral vasculature. These vascular changes may comprise increased vascular resistance, pro-thrombotic status, stroke, and microbleeds, while reductions in capillary density, cerebral autoregulation, and CBF are found. These features can accelerate the development of white matter lesions and cerebral atrophy, which eventually increase the risk of developing mild cognitive impairment and dementia. Recent focus is therefore on the (cardio)vascular risk factors like atherosclerosis, hypertension and obesity, diabetes type II etc, being modifiable via

changes in lifestyle such as diet. The Mediterranean diet containing fish, olive oil and nuts as important lipid containing components has been shown to be a strong protective factor against hypertension, obesity, stroke and Alzheimer disease, inhibiting neuroinflammation and restoring cerebral bloodflow. Contrarily, diets high in saturated fat lead to obesity and cause cerebrovascular dysregulation which is a feature of cerebrovascular pathologies, such as stroke, but also of neurodegenerative conditions, such as Alzheimer's disease. In this talk the capacity of nutrients to affect cerebrovascular function and brain function and structure in both mice and men will be reviewed.

Does a Multi-Nutrient Diet have similar beneficial Effects on Stroke in Female Mice as it has in Male Mice?

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Stroke can affect females very differently from males, and therefore preclinical research on underlying mechanisms and the effects of interventions should not be restricted to male subjects, and treatment strategies for stroke should be tailored to benefit both sexes. Previously, we demonstrated that a multinutrient intervention (Fortasyn) improved impairments after ischemic stroke induction in male C57Bl/6 mice, but the therapeutic potential of this dietary treatment remained to be investigated in females. We now induced a transient middle cerebral artery occlusion (tMCAo) in C57Bl/6 female mice and immediately after surgery switched to either Fortasyn or an isocaloric Control diet. The stroke females performed several behavioral and motor tasks before and after tMCAo and were scanned in an 11.7 Tesla MRI scanner to assess brain perfusion, integrity and functional connectivity. To assess brain plasticity, inflammation and vascular integrity, immunohistochemistry was performed after sacrifice of the mice. We found that the multinutrient intervention had diverse effects on the stroke-induced impairments in females. Similar to previous observations in male stroke mice, brain integrity, sensorimotor integration and neurogenesis benefitted from Fortasyn, but impairments in activity and motor skills were not improved in female stroke mice. Overall, Fortasyn effects in the stroked females seem more modest in comparison to previously investigated stroked male mice. We suggest that with further optimization of treatment protocols more information on the efficacy of specific interventions in stroked females can be gathered. This in turn will help with the development of (gender-specific) treatment regimens for cerebrovascular diseases such as stroke.

The extra virgine olive oil phenol hydroxytyrosol as acute therapeutic strategy after ischemic stroke

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Stroke is one of the leading causes of adult disability worldwide. After ischemic stroke, damaged tissue (penumbra) surrounding the irreversibly damaged core of the infarct is still salvageable and therefore a target for acute therapeutic strategies. Mediterranean diet (MD)-style has been shown to lower stroke risk. MD is characterized by an increased intake of extra-virgin olive oil, of which hydroxytyrosol (HT) is the foremost phenolic component. HT has been shown to possess antioxidant effects, protecting the blood vessel wall against oxidative damage, and even improving cognitive performance in Alzheimer"s disease mice. This study investigated the acute effect of an HT-enriched diet after stroke on the regain of motor and cognitive functioning, and MRI parameters. Stroke mice on HT diet showed increased CBF and also more doublecortin+ cells in the lesioned hippocampus indicating a by HT augmented neurogenesis. Additionally, HT-fed mice showed increased strength in the forepaws, as well as improved short-term recognition memory accompanied by an enhanced postsynaptic density. These results suggest that a HT-

enriched diet could be beneficial to attenuate the damage after ischemic stroke and makes HT an interesting compound to be tested in future research investigating its beneficial role as a therapeutic approach in the functional and structural recovery after ischemic stroke.

Imaging biomarkers of neuroinflammation for assessment of immunomodulatory therapies – focus on dietary interventions

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The post-ischemic inflammatory reaction is an important hallmark of stroke and strongly determines disease outcome. The (neuro-)inflammatory response therefore represents an interesting therapeutic target for neurorestorative therapies beyond classical recanalization approaches.

Novel (dietary) interventions have already shown their potential in modulating the inflammatory response in preclinical stroke models. Non-invasive molecular imaging provides early and longitudinal *in vivo* information of the spatio-temporal profile of (neuro-)inflammation and potential immunomodulatory effects of these novel interventions. This presentation will highlight the application of novel and existing molecular imaging biomarkers to assess novel immunomodulatory interventions in stroke with emphasis on dietary interventions. The integration of imaging biomarkers in the drug development pipeline may support the development and application of novel therapeutic compounds.

Brain imaging

Influence of Co-Morbidities on Post-Stroke Angiogenesis determined by imaging techniques

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Questions: During the last three decades, although there has been a large effort to understand the pathophysiology of cerebral ischemia, none of the drugs and neuroprotective strategies useful in experimental studies have succeeded clinically. To increase the translation to humans, the Stroke Therapy Academic Industry Roundtable (STAIR) has recommended to consider different stroke risk factors and imaging techniques in experimental studies (Fisher et al. Stroke 2009;40:2244-50). As well as acute treatment, the study of long term post-stroke neurorepair mechanisms, such as angiogenesis, may offer new opportunities of treatment with a broader therapeutic window. Angiogenesis, a process increased after cerebral ischemia around the affected brain area, is reduced by risk factors such as age and obesity (Petcu et al. J Angiogenes Res 2010;2:26; Heida et al. J Am Coll Cardiol 2010; 55:357-67). Dynamic enhanced-contrast imaging (DCE-MRI) is an imaging technique widely used in cancer disease to study angiogenesis, but poorly explored in the stroke field (Lin et al. J Cereb Blood Flow Metab 2008,28:1491-501). Our purpose was to study the influence along the time after experimental ischemia of different stroke risk factors on infarct volume, blood brain barrier (BBB) damage, angiogenesis/vasculogenesis and neurological outcome. We also analyzed the angiogenesis and vascular functionality after stroke, using a non-invasive technique, the DCE-MRI.

Methods: Twenty month-old corpulent (JCR:LA Cp/Cp, a model of atherosclerosis and obesity) and lean rats were used. Experimental stroke was induced by transient MCAO (90 min). Post-stroke angiogenesis was analyzed by DCE-MRI and confirmed histologically at 3, 7 and 28 days after tMCAO using a Bruker Biospec BMT 47/40 system (Bruker, Ettligen, Germany) operating at 4.7 T with a homemade surface coil. Infarct volume and BBB damage were analyzed by MRI and histological techniques. Finally, vasculogenesis was evaluated by the determination of endothelial progenitor cells (EPCs) in peripheral blood by flow cytometry and evaluating their pro-angiogenic properties in culture.

Results and Conclusions: Our results demonstrate that co-morbidities increase the infarct volume, BBB damage and impair the stroke outcome, measured by histology, MRI techniques and neurological test. Stroke risk factors impair the post-stroke angionesis and vascular function measured by histology and by DCE-MRI and also these co-morbidities impair the process of vasculogenesis, by reducing the migration of EPCs to the lesion site and by impairing their pro-angiogenic properties measured *in vitro*. Also, DCE-MRI appears as a potential non-invasive technique to evaluate in stroke patients, the vascular function and angiogenesis processes.

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Multispectral-optoacoustic-tomography imaging of acute cerebral hypoxia and matrix-metalloproteinase activity in a mouse model of cerebral ischemia and reperfusion

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Question: Hemodynamic alternations and the subsequent inflammatory responses such as matrix metalloproteinases (MMPs) upregulations play important roles in the pathophysiology of cerebral ischemia. In this study we aimed to detect *in vivo* changes in cerebral tissue oxygenation and MMP activity during and after transient middle cerebral artery occlusion (tMCAO) in mice using multispectral optoacoustic tomography (MSOT) co-registered with magnetic resonance imaging (MRI) to derive information on the ischemic lesion.

Methods: C57B6L/J mice underwent a tMCAO or sham surgery (n = 39) and were imaged by MSOT for cerebral hemodynamic changes during 1 h tMCAO or at 48 h after reperfusion. Brain MMP activities were detected by using MSOT with a MMP-activatable probe at 48 h after reperfusion [1]. To identify the affected brain regions diffusion weighted imaging and T2-weighted MRI were performed at 7 T. The MSOT deoxy-, oxyhemoglobin and MMP images were co-registered with structural MR for lesion delineation and anatomical references. *Ex vivo* near-infrared imaging and triphenyltetrazolium chloride staining were performed with brain slices for validation of MMP signal and ischemic lesions.

Results: With our system tissue oxygenation can be detected in the cortical layers up to 3-4mm of depth. Reduced ipsi/contralateral ratio of tissue oxygen saturation was observed during acute tMCAO compared to sham-operated mice (52.5 ± 23.1 %, *vs* 98.6 ± 18.3 %, p = 0.0003), which recovered to normal at 48 h after reperfusion (99.9 ± 9.4 %, n = 9). Increased ipsi-/contralateral MMP signal was detected at 48 h after reperfusion in the ipsi-lesion brain regions of tMCAO (4738.7± 2867.8 MSOT a.u., n = 5) compared to sham-operated mice (1138.4 ± 709.7 MSOT a.u., n = 4, p = 0.0479). *Ex vivo* near-infrared fluorescence imaging demonstrated increased MMP signals in the core of the ischemic lesion as defined by triphenyltetrazolium chloride staining.

Conclusion: In conclusion, MSOT constitutes a useful tool for *in vivo* visualization of hemodynamic alternations and MMP activity. We demonstrated acute cerebral hypoxia and subsequent increase in MMP activity in the mouse brain after focal cerebral ischemia with reperfusion *in vivo*.

PET imaging of stroke-associated neuroinflammation by Alpha7-nAChR ligand [18F]DBT10

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Questions: Neuroinflammatory processes after ischaemic stroke represent promising therapeutic targets. Alpha7-nicotinic acetylcholine receptors (nAChRs) play also key regulatory roles on microglia. However, in vivo imaging of neuroinflammation in acute and/or subacute stroke stages was hardly possible. Thus, the novel alpha7-nAChR ligand [18F]DBT10 was investigated for its potential to visualise post-stroke neuroinflammation.

Methods: The left middle cerebral artery was permanently occluded in 10 adult Merino sheep (pMCAO, day 1). In addition, controls (n=3) received no or sham surgery. Simultaneous dynamic (120 min) brain PET/MRI (Siemens Biograph mMR, ~300 MBq [18F]DBT10) was conducted prior to (baseline) as well as 4 hours, 7 days and 14 days after pMCAO. Parallel [15O]H2O PET and standard stroke MRI sequences were employed to compute different compartments of the ischaemic altered brain. The brains were objected to ex vivo autoradiography (AR) and histopathology immediately after PET/MRI on day 14.

Results: [18F]DBT10 was metabolized with a half-life of 18 min in blood and showed a fast cerebellar washout. The accumulation in cortical areas reached a plateau at 60 min p.i. Control animals did not show any neuropathological abnormalities. After pMCAO, the uptake of [18F]DBT10 was decreased in the ischaemically altered brain area on day 1 (SUVRs110-120min p.i. = 0.94 ± 0.04) and day 7 (0.90 ± 0.17). The tracer uptake was strongly increased in the infarction border at day 14 (1.82 ± 0.72; $p < 0.01$). The PET results on day 14 were confirmed by AR and showed a correlation with the histopathologically defined activation of microglia and infiltration of macrophages.

Conclusion: [18F]DBT10 is a promising candidate for in vivo monitoring of neuroinflammation in patients with ischaemic stroke by PET. Further, tracing the alpha7-nAChRs may prospectively contribute to assess the impact of potential therapeutic options in stroke research.

Atlas registration for MR neuroimaging in mouse models stroke: deformation-based morphometry and lesion-symptom mapping

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Questions: Image registration to a brain atlas in common space is required for modern MR neuroimaging data analyses such as automated volume of interest (VOI) analyses, voxel-based group statistics, connectomics, or for

voxel-wise correlation of behavioral data to imaging data known under the term lesion-symptom mapping. Common standards are still rare in experimental MR research, which is mostly carried out in mice. Here, we present two applications of ANTX, an in-house MATLAB toolbox for nonlinear registration of in vivo mouse brain MR images to the Allen brain atlas (ABA) including examples of how this can be used to better understand morphological and behavioral changes after stroke in the mouse.

Methods: C57/Bl6 mice were tested in the staircase test up to 3 wks after 45min of middle cerebral artery occlusion (final n=17) or a sham procedure (final n=17). Animals underwent T2-weighted MRI 24 h post surgery at 7T (Bruker BioSpin, Ettlingen, Germany) and images registered to the ABA using ANTX based on the toolboxes SPMMouse and elastix. The transformation deformation field was compared voxel-wise between groups to characterize tissue swelling/compression. For lesion-symptom mapping, the deficit in the staircase was correlated over animals in each ABA region with percent lesion volume or, in a voxel-based approach, correlated with MR image intensity. To validate this, neuronal cell density was automatically quantified on NeuN stained tissue sections acquired after 3 wks using CellProfiler (http://cellprofiler.org/), the ABA was registered to these sections using in-house MATLAB scripts, and correlation analysis of cell densities and staircase deficit was performed over animals for each atlas region.

Results: Deformation-based morphometry could characterize edema-induced swelling and compression voxel-wise. This was validated on a whole brain level by excellent correlation with previously reported edema-correction schemes on MR (Pearsson r=0.976, p=2e-11) and histology images (r=0.7, p=0.01). Lesion-symptom mapping on T2-weighted MRI 24h post stroke and neuronal densities derived from tissue sections 21d post surgery revealed that ischemic damage to somatosensory regions specifically in layer 5+6, to thalamic regions, and to CA3 in the hippocampus were highly correlated with the behavioral deficit.

Conclusions: MRI ABA registration and voxel-based group statistics are powerful tools for exploratory stroke research, e.g. to quantitatively describe edema-induced tissue changes and to identify anatomical regions whose function is important for behavioral outcome.

Multisensory stimulation improves functional recovery and resting-state functional connectivity in the mouse brain after stroke

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Introduction: Ischemic stroke causes disruption of both local and remote functional neuronal networks. Spontaneous recovery of function after stroke can be enhanced by rehabilitative strategies, which provides experience-driven cell signaling in the brain that promotes plasticity. Subjecting rodents to multimodal stimulation by housing them in an enriched environment (EE) enhances experience-dependent plasticity and functional recovery after stroke. At the systems level, functional neuroimaging in humans and rodents has revealed that recovery of sensorimotor function is associated with changes in patterns of resting-state functional connectivity (RS-FC) within and across resting-state networks. At the molecular level, GABAergic inhibitory interneurons modulate brain function and plasticity. Among this cell-type, a decrease in parvalbumin (PV)-immunoreactivity has been associated with improved behavioral outcome following experimental stroke. The aim of this study was to investigate the effect of EE on RS-FC dynamics and tactile-proprioceptive function in mice during stroke recovery, and if EE-related changes in RS-FC were associated with changes in levels of PV-expressing neurons.

Methods: Photothrombotic stroke was induced in the left sensorimotor cortex. Two days after stroke, mice were housed in standard environment (STD) or EE for 12 days. Tactile-proprioceptive was assessed using the paw placement test. Cortical RS-FC was studied using resting-state Optical Intrinsic Signal (OIS) imaging.

Results: Housing in EE significantly improved lost paw-placement function compared to mice housed in STD environment, without affecting the size of the infarct. Stroke followed by STD housing induced a marked reduction in inter- and intra-hemispheric RS-FC in several peri-lesional and remote brain regions. Housing in EE partially restored interhemispheric homotopic RS-FC between spared motor regions, in particular between posterior secondary motor cortices. Compared to mice housed in STD cages, EE exposure lead to increased RS-FC between posterior secondary motor regions and contralesional posterior parietal and retrosplenial regions. Regional RS-FC significantly correlated with decreased PV-immunoreactivity in the contralesional posterior motor region after stroke.

Conclusions: Experimental stroke and subsequent housing in EE induces dynamic changes in RS-FC in the mouse brain. Multimodal stimulation associated with EE enhances RS-FC among distinct brain regions relevant for recovery of sensorimotor function and specifically in and between regions involved in integration of multisensory input and control of movement that may involve PV/GABA interneurons. Targeting neural circuitry involving these regions by neuromodulation and multimodal sensory stimulation may improve rehabilitation after stroke.

*Shared senior authorship

Increased Capillary Transit Time Heterogeneity Is a Major Contributor of Infarction of the Ischemic Penumbra

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We examined whether per-ischemic disturbances in capillary flow patterns during ischemia contribute to tissue hypoxia and penumbral infarction. This was tested in an animal model of permanent ischemic stroke. Experiments were approved by the Danish Inspectorate.

Thirty-eight male Sprague-Dawley rats were randomly divided into either control (n=17) or 4h permanent middle cerebral artery occlusion (MCAO, n=21). For penumbra identification, laser speckle contrast imaging was applied in 9 animals (MCAO=6, c=3). In another 15 animals, a cranial window was implanted (MCAO=8, c=7). Atwo-photon microscopy-based bolus trackingwas used to quantify microvascular flow patterns by mean transit time (MTT) and capillary transit time heterogeneity(CTH) of erythrocytes every 30 min for the 4h experimental period. In a third group of animals, pericytes were visualized by Nissl dye and single capillaries were scanned for velocities of erythrocytes and diameter in-between and at the position of pericytes (MCAO = 7, c=7).

The ischemic penumbra, defined as perfusion reduction of 50-75%, shrank during the 4h occlusion period due to infarction of the penumbra. MTT and CTH was 2.580.06 s and 0.990.03 s in controls and stayed constant. In stroke animals, MTT and CTH increased maximal at 120 min following occlusion (MTT=5.480.18 s, CTH=1.470.03 s) showing a heterogeneous blood flow pattern contributing to the ischemic event. After 210 min, MTT and CTH were reduced to control equivalent values due to collapse of the capillary network. In stroke animals, single capillaries showed stalled flow at the site of pericytes indicating an essential role in stroke pathology.

Brain hemodynamics in awake mice

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Changes in brain hemodynamics accompany neuronal activation, and the majority of these effects have been examined in anesthetized rodents. However, anesthesia affects the cerebral autoregulation. In this project, we evaluated steady-state hemodynamics and oxygenation in the brain barrel cortex in awake mice. The experiments were approved by the Danish Animal Inspectorate. Imaging was performed in head-restrained C57BL/6 mice (n=6),

through a chronic sealed cranial window centered over the C2 whisker barrel. We measured intravascular oxygen partial pressure (ptO2) and mean transit-time (MTT) using two-photon microscopy. Additionally, we quantified the relative changes in cerebral blood flow (CBF) and ptO2 during functional activation by whiskers stimulation (10s). During steady state, MTT was 0.46 ± 0.14 s, arterial ptO2 93.8 ± 17.9 mmHg, venous ptO2 41.1 ± 3.6 mmHg, and the estimated oxygen extraction fraction (OEF) was 0.55 ± 0.07. During functional activation, CBF significantly increased by 6.8 ± 4.0%, and ptO2 increased in both the arterial and venous network by 2.7 ± 3.0% and 8.6 ± 2.6%, respectively. The estimated OEF showed a decrease of 12.4% ± 2.9% during functional activation. Our study describes brain hemodynamics in awake mice by the use of a combination of several optical imaging techniques. The suggested combination of optical imaging techniques can be applied to various murine disease models to evaluate the effect of the pathology on brain oxygenation and hemodynamics.

Neuroinflammation II

Targeting key signaling proinflammatory factors as a way to control microglial activation and induction of neuroinflammation

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Neuroinflammation is co-occurring phenomenon during pathological processes in the nervous system. Key player in this process is microglia. As sensing cells, microglia recognize any morbid changes. Moderate activation of microglia is beneficial, however excessive one leads to more severe degeneration of tissue and inhibition of its endogenous regeneration. One way to prevent this situation is to modulate or inhibit microglia activation.

Aim of this study was to use gene silencing technique to influence microglial activation. By targeting key proteins - NF-κB, MyD-88 and TRIF, we intended to decrease inflammatory signaling network.

Gene silencing was optimized on stable murine microglia BV-2 cell line. To induce their activation, BV-2 cells were exposed to lipopolysaccharide (LPS). Before stimulation, cells were transfected with designed siRNA sequences. Efficacy of transfection was assessed by evaluating expression of NF-κB, MyD-88, TRIF as well as IL-1β, IL-6, TNF-α, TREM1, TREM2 at mRNA (qPCR) and protein level (Western blot). Optimized sequences of siRNA were then used on primary neuronal cultures isolated from adult mice in same scheme.

Our results showed that siRNA can successfully inhibit activation of microglia *in vitro* after stimulation with LPS. Significant decrease was observed in expression of signaling proteins. However, depending on targeted factor, different decrease patterns were observed for IL-1β, IL-6 and TNF-α. Thus, mixture of siRNA was combined to achieve most successful effect.

Our results provide a potential new method to successfully limit microglia activation with siRNA technique. This approach will be further tested in *vivo,* in our models of Parkinson's disease and hypoxia-ischemia encephalopathy, in which severe inflammation is observed.

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Dedicated volumetric analysis of the spatiotemporal interaction between [18F]DPA-714 and [18F]BR-351 radiotracers in an ischemic mouse model

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Introduction: *In vivo* positron emission tomography (PET) using [^{18}F]DPA-714 and [^{18}F]BR-351 allows non-invasive assessment of the spatiotemporal expression of the translocator protein (TSPO) and matrix metalloproteinases (MMPs) respectively. We previously indicated MMPs preceded microglial activation while the spatial interaction of both imaging biomarkers has still to be elucidated.

Therefore, we aimed (i) to assess the temporal distribution of [^{18}F]DPA-714 and [^{18}F]BR-351 and (ii) to investigate their spatial interaction in a tMCAO mouse model using a dedicated volumetric analysis.

Methods: As described by Zinnhardt et al. (2015), a total of n=28 C57BL/6 mice underwent combined PET-CT and subsequent MR imaging for both [^{18}F]DPA-714 and [^{18}F]BR-351 to assess microglia activation and MMP activity 24-48 hours (n=8), 7 ± 1 days (n=7), 14 ± 1 days (n=6) and 21 ± 1 days (n=7) after a 30 min tMCAo. In the current work, the same dataset was analysed using an improved volumetric approach: after PET-CT imaging data co-registration, an ipsilesional hemisphere atlas based VOI was applied and thresholded by the mean uptake + 2.5*standard deviation (sd) of contralateral striatum. Mean lesion-to-contralateral (L/C) ratios, percentages of overlap and exclusive tracer uptake areas were determined.

Results/Discussion: [^{18}F]BR-351 uptake significantly increase as early as day 7 (ANOVA, L/C: 2.08±0.18, *p=0.043*) while [^{18}F]DPA-714 uptake peaked at day 14 (ANOVA; L/C: 2.17 ± 0.41; *p=0.011*). Voxel numbers evidence a more extended microglial activation compared to MMPs (paired t-test, [^{18}F]BR-351: 190 ± 164, [^{18}F]DPA-714: 488 ± 288, *p<0.001*).

Tracer volumes showed a constant overlap of 14.0 ± 14.2% over time (ANOVA, *p=0.255*). Analysis of exclusive tracer uptake areas suggest both mechanisms to be partly independent: 82.4 ± 16.1 % of the total [^{18}F]DPA-714 volumes were exclusive and non-overlapping with [^{18}F]BR-351, whereas 50.5 ± 33.6% [^{18}F]BR-351 volumes were exclusive for [^{18}F]BR-351.

Published *in vitro* data already suggested partly differential expressions but this has never been tracked by *in vivo* PET imaging.

Conclusion: We investigated the *in vivo* spatial dynamics of MMPs and microglia activation using a dedicated volumetric analysis. Our results indicated a primary MMP activation independent of microglia while later expression may be sustained by activated microglia in specific overlapping regions of the damaged tissue. Still, additional lesion extend depicted by T2w MRI must be included in the analysis.

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A high-dimensional immunological map of the peripheral blood compartment in stroke patients

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Introduction: Infectious complications post-stroke pose a significant barrier to recovery and increase morbidity and mortality. Studies have endeavoured to underpin basis of increased infection susceptibility by investigating alterations to systemic immunity but focus has been largely restricted to examining the roles of either the innate or adaptive arms in isolation of each other. As a result of these approaches, there is a lack of translation and consensus on the precise alterations to systemic immunity post-stroke.

Methods: Therefore, in a clinical setting, we aimed to characterise the spectrum and temporality of immune alterations in circulation following cerebral ischaemia and the effect of the interleukin-1 receptor antagonist (IL-1Ra) over 72 h. To address this, we employed traditional flow cytometric and high-dimensional analyses by implementing the visual *t* distributed stochastic neighbour embedding (viSNE) algorithm on our data set.

Results: We determined that stroke decreased the frequency of conventional dendritic cells 1 (cDC1), cDC2s and non-classical monocytes over 72 h, possibly ascribed to an increase in the number of classical and intermediate monocytes. viSNE analyses also identified heterogeneity in the surface phenotype of classical monocytes (increased CD14 and decreased HLA-DR) that could modulate their functional capacity. Further, stroke also increased the frequency of CD27+IgD+CD38+ memory B cells, an important early source of secreted IgM during infections but decreased CD1c, a non-classical antigen-presenting molecule expression on B cells. Whilst stroke did not modulate the frequency or numbers of T lymphocytes, viSNE identified an upregulation of markers associated with their exhaustion. In all instances, there were no consistent effects of IL-1Ra

Conclusions: We are the first to present a comprehensive immunological map of alterations to peripheral immune networks post-stroke and determine their temporality. Overall, we demonstrate that cerebral ischaemia imposes complex alterations on innate an adaptive immune networks that could impair the generation of antigen-specific T cell responses and as a result, increase infection susceptibility in patients.

B cells migrate to remote brain areas supporting functional recovery after stroke

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Question: Stroke induces significant immune cell infiltration into the brain. Recent studies demonstrate a protective role for some leukocyte subsets through the production of anti-inflammatory cytokines. In fact, the early presence of B cells coincides with reduced inflammation, and B cell depletion impedes neurogenesis, increases anxiety, and exacerbates memory deficits in post-stroke mice. These deficits are mediated by areas typically outside of the infarct. Therefore, **we hypothesized that B cells migrate into regions outside the ischemic penumbra to promote post-stroke neuroplasticity and functional recovery.**

Methods: Splenic B cells from adult male human CD20 (hCD20)–null mice (B6 background) were labeled with eFluor450 proliferation dye. Rituximab (hCD20$^+$ depleting drug) was given for 3 days to hCD20$^+$ recipient mice prior to 60-min transient middle cerebral artery occlusion (tMCAo). Recipient mice received 5x10^6 eFluor450$^+$ B cells (n=4) or PBS (n=3) intravenously at 7, 24, 48, and 72h after tMCAo, and tissues were collected 96h post-tMCAo. Flow cytometry assessed eFluor450$^+$ B cell migration in secondary lymphoid organs. Serial two-photon tomographic (TissueCyte 1000) volumetric images were co-registered with the Allen Institute for Brain Science Common Coordinate Framework (v3.0) via the open-source "NiftyReg" software package. B cells were identified via machine-learning algorithms using the open-source software package "Ilastik." eFluor450$^+$ B cell signal was normalized to stroke-induced autofluorescence (i.e. PBS controls) and analyzed by student's t-test or ratio-paired parametric t-test for ipsilesional (i.e. ipsi) and contralesional (i.e. contra) egress (GraphPad Prism). Data shown as % mean endogenous fluorescence and significance was $p<0.05$.

Results: eFluor450$^+$ B cells were found in spleens and cervical lymph nodes of recipient mice. 3D brain reconstruction showed infarcted brain regions depicted by diffuse autofluorescence. eFluor450$^+$ B cells exhibited bilateral, brain region-specific migration after stroke to 11 of 41 areas identified for roles in functional recovery. B cells migrated into cerebral cortex (ipsi: 260%, $p<0.05$; contra: 229%, $p<0.05$), dentate gyrus (ipsi: 254%, $p<0.07$; contra: 182%, $p<0.05$), hypothalamus (ipsi: 224%, $p<0.05$; contra: 292%, $p<0.05$), and cerebellum (ipsi: 237%, $p<0.07$; contra: 151%). The latter areas were outside of the identified infarct, though only the cerebellum exhibited preferential B cell recruitment to the ipsilesional hemisphere ($p<0.05$).

Conclusions: Our data show that B cells express region-specific migration within the brain 96h after stroke. The brain regions identified using serial two-photon tomography regulate motor and cognitive functions such as motor coordination, neurogenesis, learning, and memory formation, all deficits exhibited by B cell-depleted mice after stroke. Future studies will determine if the spatial location of B cells indeed supports post-stroke functional recovery.

Astrocytes express fibrin chains immunoreactivty.

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Subarachnoid hemorrhage (SAH) results in permanent physical and cognitive disorders and accompanied by severe slowing of CSF flow. CSF flow block may be partially reversed by tPA or tissue factor (TF, FIII) antibodies suggesting an important role of fibrin deposition in subarachnoid space (SS).

We observed fine fibrin deposition along the paravascular spaces in naive animals, which increased dramatically following SAH. Astrocytes (Astro) may regulate the CSF flow as their TF expressing endfeet form *glia limitans* (GL) and sheath of intracerebral vessels. Following SAH, fibrin deposits in the areas beyond the hemorrhage. Traditionally it is thought that fibrin(ogen) enters the SS through damaged blood brain barrier or vessels. However, deposition of fibrin remotely from hemorrhage suggests that fibrin chains (FC) Aα, Bβ and g can originate in the brain. Here we demonstrate *in vivo* and *in vitro* that astroglia expresses FCs immunoreactivity (IR).

SAH in mice was induced by the filament perforation of the circle of Willis. Four days after SAH animals were anesthetized, transcardially perfused and fixed. Whole brain was processed for immunofluorescent (IF) analysis of fibrin(ogen) deposition on the brain surface or brains slices. Coronal slices were processed for immunohistochemical detection of fibrin(ogen) and FC Aα, Bβ, g and GFAP.

Normal human Astro were grown in BSA-free media to confluency and stimulated with NOC-18 (100 µM), TNF-α (100 nM), ATP-g-S (100 µM) for 4 hours. Culture was fixed and washed/permeabilized with 0.1% Triton and processed for IF.

Four days following SAH FC Aα IF associated with GL increased 3.2 and 2.5 times ($p<0.05$ and $p<0.01$) on the ventral and dorsal brain surfaces respectively; FC Bβ increased by 3 times ($p<0.01$) on the dorsal surface and FC g increased by 3 times ($p<0.01$) on the ventral surface compared to sham animals.

Human cultured Astro constitutively expressed all three FCs. The FC expression differentially changed when exposed for 4 hours to biologically significant stimuli: TNFα, NO or ATP (Table).

Table. Changes in specific FCs IF (arbitrary units/cell) in cultured Astro in response to exposure to TNFα, NO-donor (NOC-18) and stable ATP analogue (ATP-g-S).

	FC Aα				FC Bβ				FC γ			
Control	1.00	±	0.07		1.00	±	0.12		1.00	±	0.13	
TNFα	1.48	±	0.21	*	0.92	±	0.14		0.79	±	0.04	
NOC-18	1.41	±	0.18	*	1.14	±	0.20		0.89	±	0.08	
ATP-γS	0.82	±	0.12		0.58	±	0.11	*	0.66	±	0.07	*
*** p < 0.01**												

We demonstrate for the first time that Astro express FCs IR suggesting potential presence of endogenous to the brain FCs differentially changing to biologically significant stimuli. SAH is followed by increased expression of FCs associated with GL remote from the hemorrhage. Fibrin deposition may play an important role in CSF flow regulation. There is a possibility that individual FCs may have other physiological roles. We conclude that brain/astrocytes are capable of production of FCs, which may be involved in various normal and pathological processes such as regulation of CSF flow and brain pathology.

Interleukin-1 receptor antagonist treatment after stroke does not alter systemic markers of anti-microbial defence

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Introduction: Infection is a common complication after stroke that significantly affects overall recovery and outcome. Deficits in immune function are proposed to contribute to infection susceptibility after stroke. We have previously shown that experimental stroke induces a loss of innate-like splenic marginal zone B cells responsible for early production of the immunoglobulin IgM after infection onset and reduced IgM was associated with the development of pneumonia. Reduced circulating IgM was also measured in stroke patients. Blockade of the cytokine IL-1 is a candidate treatment for stroke, which acts by reducing harmful inflammatory responses in the brain. As IL-1 blockade can reduce immune system activation, this treatment could further compromise systemic immune suppression after stroke increasing the risk of infection in patients. Furthermore, IL-1b signalling is thought to regulate protective IgM antibody responses of certain subsets of innate-like B cells. We aimed to determine if treatment with the recombinant IL-1 receptor antagonist (IL-1Ra) after stroke further compromised concentrations of immunoglobulins and associated immune molecules known to be impaired after both experimental and clinical stroke

Method: We assessed levels of immunoglobulins, complement components and noradrenaline in stroke patients treated with IL-1Ra in comparison to placebo-treated and non-stroke controls using ELISA and multiplex cytokine and immunoglobulin assays.

Results: We found stroke was associated with reductions in IgM, IgG1, IgG4 and IgA antibodies, but this was not influenced by treatment with IL-1Ra. Complement components were differentially affected by stroke, however components associated with the classical and lectin pathways of complement activation were upregulated, suggesting activation of these complement pathways after stroke. Treatment with IL-1Ra did not influence the effects of stroke on complement concentrations. Noradrenaline concentrations were increased after stroke and this correlated with stroke severity, but again treatment with IL-1Ra did not alter this.

Conclusions: Overall, treatment with IL-1Ra after stroke does not alter immunoglobulin and complement concentrations after stroke and is unlikely to further contribute to infection susceptibility through these mechanisms. Furthermore, treatment with IL-1ra showed no effect on circulating noradrenaline levels after stroke.

Multiple roles for TREM2 in the development of and microglial reaction to neuronal injury

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Background / question: Triggering Receptor Expressed on Myeloid cells 2 (TREM2) is an innate immune receptor expressed by microglia with key roles in the regulation of central nervous system (CNS) homeostasis and neuroinflammation. Mutations in the TREM2 gene are known to contribute to a range of neurological conditions demonstrating the importance of microglial dysfunction and neuroinflammation in neurodegenerative processes. The precise function of TREM2 in mediating microglial responses to CNS injury remains unclear, particularly in the context of ischaemic and neuronal insults. We therefore investigated the impact of TREM2 deficiency on microglial responses and CNS pathology using a model of global forebrain ischaemia in which delayed neuronal injury is induced.

Methods: Wild type (C57Bl/6) and TREM2-/- male mice underwent bilateral common carotid artery occlusion for 20 min and were recovered for 3 days.

Results: Histological analysis demonstrated diffuse neuronal injury most frequently observed in the hippocampus accompanied by robust microglial activation. Reduced neuronal loss was evident in TREM2-/- mice suggesting TREM2 may contribute to neuronal damage in early stages of ischaemic insult. In addition to this, TREM2 deficiency was associated with reduced microgliosis and impaired invasion into areas of neuronal damage. No difference in Ki67 expression was observed between genotypes suggesting this reduced microgliosis was not due to impaired proliferation. Preliminary findings also suggest lack of TREM2 deficiency impaired microglial phagocytosis of damaged neurons in response to global ischaemia. Furthermore, although RT-QPCR analysis revealed increased expression of inflammatory markers in response to global ischaemia there were no significant differences observed between genotypes.

Conclusions: Taken together, these data suggest TREM2 contributes in multiple time-dependent ways to both the development of and reaction to neuronal with key roles in the regulation of injury-induced microglial migration and phagocytosis.

Stroke and brain hemorrhage

Folic Acid Exerts Post-Ischemic Neuroprotection *In Vitro* Through HIF-1α Stabilization

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Objective: The increasing prevalence of ischemic stroke coupled with the lack of effective treatment highlight the pressing need for a continued research to discover safe and effective neurotherapeutics that positively influence the pathophysiological pathways and extend the benefit to a larger number of stroke patients. A multi-target drug repurposing strategy identified Folic Acid as hypoxia-inducible factor-1α (HIF-1α) stabilizer and its post-ischemic neuroprotective efficacy was evaluated *in vitro.*

Methods: Structure-based drug docking and molecular dynamics simulation approaches were adopted initially to screen FDA-approved drug database against PHD2, FIH, and pVHL, the three regulatory proteins of HIF-1α. Physical binding of the top common hit compound with the proteins is verified using Drug Affinity Responsive Target Stability (DARTS) assay. Stabilization of HIF-1α and its nuclear localization in the presence of top hit molecule were studied using HIF1α-GFPSpark tag vector system. Post-ischemic neuroprotective efficacy of the hit molecule was evaluated using oxygen glucose deprivation (OGD) model. The effect of treatment of screened compound on downstream genes of HIF-1 was studied using real time PCR. Finally, the pro-angiogenic capability of the compound was evaluated using chick chorioallantoic membrane (CAM) assay.

Results: Combination of computational and experimental studies identified Folic Acid (FA) as the multi-target (PHD2, FIH, and pVHL) stabilizer of HIF-1α from the FDA drug database. The HIF1α-GFPSpark tag vector transfection assay demonstrated the positive effect FA on HIF-1α stabilization and its nuclear localization. Post-ischemic (1 hour) treatment of SH-SY5Y cells subjected to 5 hours of oxygen glucose deprivation (OGD) followed by reoxygenation indicated that 0.05 µM FA induced highest and statistically significant cell viability (9.33%; $p < 0.05$). Further, the neuroprotective mechanism of FA was elucidated by measuring the expression of BAX, NFE2L2, VEGF, and EPO genes in a time-dependent manner (5 and 11 h following FA treatment). VEGF and EPO expressions were significantly increased by 5.41- and 1.35-folds, respectively, whereas BAX expression reduced by 4-fold at 11 h post-FA treatment. NFE2L2 expression was elevated (1.65-fold) at 5 h with no major difference at 11 h post-FA treatment. The chicken chorioallantoic membrane (CAM) assay demonstrated the pro-angiogenic potential of FA as evidenced by an increased blood vessel density and branching.

Conclusion: The present study elucidates for the first time that the postischemic neuroprotection exerted by FA may be attributed to its HIF-1α stabilization and pro-angiogenic properties.

Targeting cerebral oedema and intracranial pressure in an ovine model: the NK1 receptor antagonist as a novel therapy

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Question: Cerebral oedema and elevated intracranial pressure (ICP) are the leading cause of death in the first week following stroke. The deleterious effect of space-occupying swelling and concomitant rise in ICP leads to secondary neurological deterioration and irreversible brain tissue damage. Despite this, current treatments are limited and fail to address the underlying mechanisms of swelling, highlighting the need for development of targeted treatments. Although the factors associated with the development of cerebral oedema remain largely unclear, neurogenic inflammation and the release of substance P (SP) following stroke has been linked to profound blood brain barrier (BBB) disruption, cerebral oedema and poor functional outcome. SP elicits its effects by binding the NK1 tachykinin receptor (NK1R), with administration of an NK1R antagonist completely ameliorating BBB dysfunction and cerebral

oedema following stroke in rodent models. However, when screening novel therapeutic agents, such as the NK1R antagonist, it is also essential to use clinically relevant large animal models to improve the likelihood of successful clinical translation. In light of this, the current study examined the efficacy of NK1R antagonist treatment in reducing cerebral oedema and ICP in a clinically relevant ovine stroke model.

Methods: Merino sheep (9M;13F; 64.70kg±7.42kg; 18-36mths) were anaesthetised and subject to 2hrs middle cerebral artery occlusion (MCAo) with reperfusion. Animals were then randomly allocated into one of the following treatment regimes: early treatment at 1-3d post stroke (1mg/kg NK1R-antagonist IV; *n*=6), delayed treatment at 5d post stroke (1mg/kg NK1R-antagonist IV; *n*=6), saline vehicle (*n*=6) or sham surgery (*n*=4). At 6d post stroke, when elevated ICP is known to be established in this model, animals were re-anesthetised and ICP measured for 4hrs, followed by MRI for cerebral oedema (DWI, FLAIR) and to confirm vascular reperfusion (MRA). Following MRI, animals were perfused under anaesthesia and brains removed and processed for analysis by immunohistochemistry.

Results: Following stroke, ICP was significantly decreased following administration of the NK1R antagonist in both the early ($p<0.01$) and late ($p<0.0001$) treatment regimes when compared to vehicle. Profound cerebral oedema was observed in vehicle treated animals at 6d, in keeping with the elevated ICP.

Conclusions: These findings provide substantial evidence that NK1R antagonist treatment is an efficacious novel therapy for the treatment cerebral oedema and elevated ICP following stroke, as it is able to both prevent increases in ICP when administered early and also reduce established ICP when administered in a delayed fashion following ovine stroke. This study provides compelling evidence for the clinical evaluation of NK1R antagonist treatment in the management of cerebral oedema and elevated ICP.

Role of neurexin-1β and neuroligin-1 in cognitive dysfunction after subarachnoid hemorrhage in rats

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Question: Neurexin-1β and neuroligin-1 play an important role in the formation, maintenance and regulation of synaptic structures. However, the potential roles of neurexin-1β and neuroligin-1 in subarachnoid hemorrhage (SAH)-induced cognitive dysfunction have not been reported.

Methods: *In vivo*, two hundred and twenty-eight Sprague–Dawley rats were used. Experimental SAH model was induced by single blood injection to prechiasmatic cistern. Primary cultured hippocampal neurons were exposed to oxyhemoglobin to mimic SAH *in vitro*. Specific siRNAs and over-expression plasmids for neurexin-1β and neuroligin-1 were exploited both *in vivo* and *in vitro*. Western blot, immunofluorescence, immunoprecipitation, neurological scoring and Morris water maze were performed to evaluate the mechanism of neurexin-1β and neuroligin-1, as well as neurological outcome.

Results: Both *in vivo* and *in vitro* experiments showed the SAH-induced decrease in the expressions of neurexin-1β and neuroligin-1 and the interaction between them in neurons. In addition, the interaction between neurexin-1β and neuroligin-1 was reduced by their knockdown and increased by their overexpression. The formation of excitatory synapses was inhibited by oxyhemoglobin treatment, which was significantly ameliorated by overexpression of neurexin-1β and neuroligin-1 and aggravated by knockdown of neurexin-1β and neuroligin-1. More importantly, neurexin-1β and neuroligin-1 overexpression ameliorated SAH-induced cognitive dysfunction, while neurexin-1β and neuroligin-1 knockdown induced an opposite effect.

Conclusion: Enhancing the expressions of neurexin-1β and neuroligin-1 could promote the interaction between them and the formation of excitatory synapses, which is helpful to improve cognitive dysfunction after SAH. Neurexin-1β and neuroligin-1 might be good targets for improve cognitive function after SAH.

ATN-161 stabilizes the blood-brain barrier and is neuroprotective after experimental ischemic stroke through inhibition of integrin α5β1

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Ischemic stroke remains a leading cause of death and disability world-wide with limited therapeutic options. Endothelial cell integrin receptors, specifically the β1 subtype, play a direct role in blood-brain barrier (BBB) dysfunction through regulation of barrier-forming tight junction (TJ) proteins.

Hypothesis: We hypothesized that α5β1, a pro-angiogenic fibronectin receptor that is highly expressed in developing brain vasculature but downregulated in the adult brain, is beneficial after stroke by modulating BBB breakdown through its effect on the localization and expression of α5β1 TJ protein, claudin-5, and inflammatory cell infiltration.

Methods: *In vivo*: 3-month-old male C57Bl/6 wildtype mice underwent distal middle cerebral artery occlusion for 1 hour. Mice were treated intraperitoneally with ATN-161 (1 mg/kg) upon reperfusion, post stroke day (PSD) 1 and PSD2. Infarct volume was assessed on PSD3 using TTC and T2 weighted MRI. Vasogenic edema was evaluated by apparent diffusion coefficient (ADC) via MRI. An 11-point behavioral battery, was performed on PSD1-14. Expression of MMP-9, claudin-5 and IL-1β levels were determined by qPCR. Immunohistochemical analysis of α5 integrin, collagen IV, claudin-5, CD31, CD45, and DAPI expression was performed on PSD3. *In vitro*: Brain endothelial cells underwent oxygen-glucose deprivation (OGD) or TNF-a treatment followed by ATN-161 (10 μM) administration. Permeability was determined by FITC dextran migration or trans-endothelial electrical resistance (TEER). Immunocytochemistry of Claudin-5 was performed.

Results: α5 integrin expression is upregulated exponentially during days PSD2 to PSD4. Acute administration of ATN-161 significantly decreased infarct volume and vasogenic edema, and improved functional recovery. Additionally, ATN-161 appeared to stabilize the BBB by increasing expression of the tight junction protein Claudin-5, while decreasing expression of extracellular matrix proteinase, MMP-9, inflammatory cytokine, IL-1β, and infiltrating leukocytes (CD45). Claudin-5 expression and monolayer permeability was conserved with ATN-161 administration following OGD and TNF-α treatment *in vitro*.

Conclusion: Administration of the α5β1 inhibitor, ATN-161, reduces infarct volume, vasogenic edema, and protects from functional deficit following experimental stroke. Furthermore, ATN-161 reduced inflammatory markers (IL-β and leukocytes) while conserving claudin-5 expression both *in vivo*and *in vitro*, with increased monolayer integrity *in vitro*, suggesting a more intact BBB. Collectively, these results suggest inhibition of α5β1 via ATN-161 could represent a promising novel therapeutic approach for ischemic stroke.

Novel thromboembolic stroke model in rat.

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Question: The aim of this study was to develop a new thromboembolic stroke model in rats that mimics the human stroke closely, and validate its clinical relevance through confirming the possibility of using the model for evaluation of thrombolytic agents.

Methods: Stroke was induced in male Wistar rats by a local injection of thrombin into the lumen of the middle cerebral artery (MCA). The cerebral blood velocity was measured by laser Doppler before the injection of thrombin (100% baseline) and throughout the duration of the experiment. The clot and drop remained stable for a minimum

of 1 h. In addition, the sham operation consisted of an empty pipette inserted into the MCA lumen with nothing injected.

The study consists of three treatment groups; (I) vehicle, (II) treated with tissue plasminogen activator (t-Pa) (3mg / kg), (III) sham. Treatment was given 1 h after stroke onset with a bolus dose of 10 % and the remaining over a duration of 40 minutes.

Functional outcome was evaluated through a 28-p neuroscore which was performed prior to the surgery, and 24 hours after stroke onset. In addition, the infarct lesion volume was evaluated by magnetic resonance imaging. Data is presented as mean ± standard deviation.

Results: In-situ thrombin injection resulted in a clot formation and cortical brain injury with a reproducible infarct lesion volume 24 h after stroke onset. Treatment with t-PA leads to recanalisation of the vessel and therefore, prevents the impairment of neurological function. A reduction in infract volume was observed after t-PA treatment (18.80 ± 19.51 mm3) compared to vehicle (93.96 ± 24.68 mm3) 24 hours after stroke onset. In addition, the neurological function was improved after t-PA treatment (25.40 ± 0.89 p) compared to vehicle (20.75 ± 1.17 p). Sham operated animals showed no indication of clot formation, infarct lesion or a reduction in neurological function.

Conclusion: Here we present a new thromboembolic stroke model in rat with a reproducible infarct lesion and impairment of neurological function, which can be prevented with treatment with t-Pa. In addition, the model closely resembles the human ischemic stroke which makes it a clinical relevant model. This model can be used to evaluate new treatments for ischemic stroke, especially in the search for new thrombolytic options.

A semiquantitative non-invasive measurement of PcomA patency in C57BL/6 mice can predict infarct size in filament MCAo

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Objective: The filament middle cerebral artery occlusion model (fMCAo) is one of the most widely used models for ischemic stroke. Numerous studies use C57BL/6 mice for fMCAo, but lesion sizes in this strain are highly variable. Consequently, large sample sizes are needed to study the effect of neuroprotective treatments on lesion size with an adequate statistical power. A known reason for the large variance of lesion volumes in this particular strain is the high variability of the posterior communicating artery (PcomA). Animals without a patent PcomA have long been known to show larger infarct sizes in the fMCAo model. To date, most studies analyzing this effect have used invasive or ex vivo methodology. In this study we aimed at providing a semi-quantitative non-invasive in vivo method to assess PcomA patency in the fMCAo model. Inclusion of the PcomA variability as a covariate could lead to better ways to discriminate treatment effects in this model.

Material/Methods: Male C57BL/6 mice were subjected to transient 45 min focal cerebral ischemia using the filament MCAo model. Magnetic resonance (MR) imaging was employed to measure lesion sizes 24 h after reperfusion. Time-of-flight (TOF) MR Angiographies obtained on a 7 T rodent MR scanner were used to obtain vessel imaging. Two independent groups were included. The first group served to develop a pipeline for image acquisition, post-processing and analysis including image registration to an atlas. This methodology was then validated in an independent group. All procedures were randomized and analysis of PcomA size was conducted blinded to lesion size.

Results: With our approach we were able to show a highly significant ($p<0.001$, Coefficient of determination $r2 = 0.55$) negative correlation of a standardised semiquantitative non-invasive measure of PcomA size and edema corrected lesion volume 24 hours after reperfusion in a 45 min fMCAo model using C57BL/6 mice. Signals over the

75th Percentile in this measurement reliably predicted small lesion sizes below the 50th percentile. The methodology derived from study group 1 yielded comparable results in the independently conducted and analysed second group ($p<0.01$, $r2 = 0.3$).

Conclusions: We have demonstrated that in vivo evaluation of PcomA patency is feasible. A semi-quantitative measure of PcomA size extracted from non-invasively acquired TOF MRA images predicts lesion size in the fMCAo model and can potentially be used as a covariate to reduce within group error variance and thus allow to more accurately assess the effect of a potentially neuroprotective treatment.

Removing the brakes on brain recovery after stroke by negative allosteric modulation of the metabotropic glutamate receptor 5

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Introduction: Stroke is a main cause of life-long disability worldwide. Whilst task-specific rehabilitative training is an evidence based stroke treatment, there is no pharmacological therapy that enhances neurological recovery after stroke beyond the therapeutic time window of neuroprotection. In the experimental setting, housing rodents in an enriched environment (EE) accelerates behavioural recovery and enhances brain-wide functional connectivity in cortical regions local and distant from the injury (Hakon et al., NeuroImage: Clinical, 2018). We investigated the importance of mGluR5 receptors in neurological recovery after stroke.

Methods: We employed the photothrombosis or endothelin-1 stroke models in rats or mice. Housing in an EE or treatment with mGluR5 modulators typically started 2 days after stroke and continued for 14 days. Neurological function was assessed by paw placement, grid, and grip tests. Resting-state functional connectivity (RS-FC) was assessed using optical intrinsic signal (OIS) imaging.

Results: Treatment with the mGluR5 NAM 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) provided a robust improvement of neurological function compared to vehicle-treated animals 14 days after stroke. Improvement was attained even when treatment started 7 days after stroke, and the therapeutic effect remained at least 7 days after termination of treatment. Housing in an EE in combination with MTEP treatment provided a synergistic recovery enhancing effect. Daily pre-treatment with the mGluR5 positive allosteric modulator VU0360172 prevented the recovery enhancing action of MTEP. In addition, the recovery enhancing effect of EE was prevented by concomitant treatment with VU0360172. At the network-level, treatment with MTEP increased intra-hemispheric RS-FC in contralesional sensorimotor areas, and at the cellular level a time-dependent modulation of mGluR5 mediated cell signalling was found. None of the treatments affected infarct size when assessed at 7 or 14 days of recovery. The presence of mGluR5 was confirmed in both brain hemispheres of stroke patients.

Conclusions: We conclude that post stroke mGluR5 activation hampers neurological recovery in rodents and that treatment with a mGluR5 NAMs provides strong recovery of integrated sensorimotor function associated with contralesional cortical remodelling. Selective mGluR5 NAMs could become a future pharmacological therapy for promoting functional recovery after stroke.

Cutting-edge brain imaging in disease and regeneration

Molecular MR imaging of neuroinflammation

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Endothelial cells of the central nervous system over-express surface proteins during neurological disorders, either as a cause, or a consequence, of the disease. Since the cerebral vasculature is easily accessible by large contrast-carrying particles, it constitutes a target of choice for molecular magnetic resonance imaging (MRI). During my talk, I will highlight the most recent advances in molecular MRI of brain endothelial activation and focus on the development of micro-sized particles of iron oxide (MPIO) targeting adhesion molecules including ivascular cell adhesion molecule 1 (VCAM-1) and P-Selectin. I will also discuss the perspectives and challenges for the clinical application of this technology in neurovascular disorders (ischemic stroke, intracranial hemorrhage, subarachnoid hemorrhage, diabetes mellitus), neuroinflammatory disorders (multiple sclerosis, brain infectious diseases, sepsis) and neurodegenerative disorders (Alzheimer's disease, vascular dementia, aging).

Brain structure-function relationships assessed with MRI

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The relationship between functional and structural connectivity strength in the brain remains uncertain. We compared high-field resting-state fMRI, diffusion-based tractography and neuronal tracer data to characterize the connectome in rat brain. Our study revealed that strong structural connectivity is not required for strong functional connectivity. Distinct structure-function relationships were found at different hierarchical levels: functional connectivity strength correlated moderately with diffusion-based structural connectivity strength, but did not significantly correlate with neuronal tracer-based structural connectivity strength. Our study underscores the importance of examining or appraising connectivity at different hierarchical levels for reliable assessment of neural network (re)organization in health and disease.

Brain imaging of vascular dysfunction in CNS disorders

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Structural and functional integrity of blood vessels and adequate supply of blood are essential to normal brain functioning. In addition, cerebral blood flow shortfalls and blood-brain barrier dysfunction are increasingly recognized aspects of neurodegenerative disorders in humans and animal models. Our advanced magnetic resonance imaging (MRI) techniques offer new possibilities to understand how the brain works in health and disease. This includes methods to detect subtle regional changes in the cerebrovascular system integrity. Novel MRI approaches are examined in combination with biomarker profiles in biofluids, behavioral testing and post-mortem tissue analysis to expand our knowledge on central nervous system pathogenesis, translating from relevant animal models to human patients with stroke or dementia.

Ultrafast Ultrasound imaging: new perspective for brain imaging

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Functional imaging modalities such as fMRI or optical imaging identify regions of brain activity by measuring changes in blood flow thanks to the neurovascular coupling. Paradoxically, Ultrasound was not present in the field of Neuroscience till recently, whereas it is the most used modality for blood flow imaging in clinics. The reason for this was the poor sensitivity of Doppler ultrasound limiting blood flow imaging to large arteries. Ultrafast Doppler imaging based on plane wave imaging breaks this barrier and enabled recently the emergence of fUltrasound (by analogy to fMRI). fUltrasound (fUS) is able to provide unique whole brain images of perfusion on small rodents with unprecedented resolutions (100 µm and 10 ms). Functional imaging of cerebral blood volume during epileptic seizures, whisker or odor stimulations, drug injection emphasizes the potential of this new imaging modality to provide completely new information for the understanding of brain. As fUltrasound relies on ultrafast ultrasound acquisitions, it also enables the whole brain mapping of vascular indexes (such as resistivity, pulsatility or transient systolic time indexes) within a single cardiac cycle in addition to functional imaging of brain activity on longer time scales.

fUS is a great tool for neuroimaging on small animals as it already helps both to answer unsolved questions and image the functional activity of to date unexplored brain regions. It should become a a full-fledged imaging modality of neuroscience as it provides the first whole brain and portable neuroimaging modality for awake and freely moving animal studies. Beyond small animal imaging, clinical fUS should become an alternative to fMRI in particular applications, such as newborns or preterm infants both for neonatal seizure monitoring or cognitive science studies. It could also become a powerful portable tool for neuroimaging during peroperative surgery on adults.

Finally, we demonstrated recently that it can be combined with 3 µm diameter microbubbles injections in order to provide a first *in vivo* and non-invasive imaging modality at microscopic scales deep into organs combined with contrast agents by localizing the position of millions of microbubbles at ultrafast frame rates. This ultrasound localization microscopy technique solves for the first time the problem of *in vivo* imaging at microscopic scale the whole brain vasculature. Beyond fundamental neuroscience or stroke diagnosis, it will certainly provide new insights in the understanding of tumor angiogenesis.

The gut-brain axis in neurological disorders and repair

The gut-brain axis in Alzheimer's disease

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An important role of the gut-microbiota-brain axis has been implicated in a number of brain diseases. We have previously demonstrated a key role of the gut microbiome in regulating the inflammatory response to acute stroke and modulating stroke outcome. Recent studies have suggested a function of the gut microbiota in modulating cerebral amyloid pathology in mouse models of Alzheimer's disease (AD). Therefore, we investigated the microbiota-brain axis by generating germfree AD mouse models. We observed a substantial reduction of amyloid plaque load in germfree animals, which was reversed by naterual recolonization of ex-germfree littermates. Reduced plaque load in germfree mice was associated with modulation of microglial activity and expression of neuroinflammatory markers. Based on our previous studies in stroke models, we hypothesized short chain fatty acids (SCFA) to be key mediators of the microbiota effect on AD pathology along the gut-brain axis. Indeed, supplementation of SCFA to germfree animals increased plaque load comparable to colonized animals and mimicked several inflammatory markers comparable to bacterial colonization. This data provides unequivocal evidence for the contribution of the gut microbiome to AD progression and reveals SCFA as the key bacterial mediator for this effect.

Acute brain injuries affect the gut microbiome

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Changes in intestinal microbiota are associated with diverse human diseases. Recent data indicate that the bi-directional communication between the microbiota and the brain might contribute to known clinical complications such as infections or paralytic ileus seen after stroke. In mice, brain damage induced by stroke or traumatic brain injury leads to altered composition of caecal microbiota, with specific changes in Peptococcaceae and Prevotellaceace correlating with the extent of injury. These effects are in part mediated by noradrenaline release from the autonomic nervous system with altered caecal mucoprotein production. Changes in intestinal microbiota are also seen in patients after acute brain inury, which may affect recovery and treatment of patients.

Intestinal immune cells in stroke immunity

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Inflammation and immune cells are major components in the pathophysiology of ischemic stroke and contribute to acute and delayed tissue injury. However, our incomplete understanding of the factors regulating the immune responses triggered by cerebral ischemia remains a significant obstacle to the development of effective therapeutic interventions based on modulating post-ischemic inflammation. Besides activation of brain resident immune cells, ischemic stroke is characterized by the recruitment of peripheral innate and adaptive immune cells that participate in the inflammatory response and contribute to the damage. Intestinal commensal bacteria, the most abundant symbiotic compartment in the body, have emerged as a potent regulator of the immune system. Remarkably, gut microbiota influence the development and proliferation of immune cells including γδT cells and regulatory T cells (Treg), which have been implicated in ischemic brain injury. These observations raise the possibility that the microbiota have the potential of modulating the outcome of cerebral ischemia. Recent efforts have elucidated some of the mechanisms underlying the modulation of the inflammatory response to ischemic brain injury by commensal bacteria. These studies have identified a network of dendritic cells (DC), Treg and γδT cells in the small intestine that is regulated by commensal microbiota. Changes in the network lead to altered immune cell recruitment after cerebral ischemia and affect stroke outcome. We found that DC isolated from mesenteric lymph nodes (mLN) of mice with altered flora were more efficient in inducing Treg than the DC isolated from mLN of control mice. In addition, we found that Treg generated in co-cultures with DC isolated from mice with altered flora inhibited IL-17+γδT differentiation more efficiently than Treg generated by DC from control mice. Using in vivo cell tracking it has been shown that distinct immune cell populations traffic from the gut to the brain after stroke, where they localize in the brain and leptomeninges. Our studies also show that the meninges function as a gatekeeper in post-ischemic inflammation. This is evidenced by the fact that γδT cells did not enter the brain after stroke, but remained restricted to the leptomeninges where their numbers increased after stroke. Because inflammatory cells might enter the injured brain through extravasation from compromised meningeal vessels, meningeal IL-17+γδT cells would be at a strategic location to control the trafficking to the brain parenchyma of monocytes and neutrophils – the main leukocyte populations found in the ischemic brain. In conclusion, commensal microbiota have emerged as an important modulator of central nervous system pathologies. Given that the intestinal microbiome is highly modifiable by diet, drugs, and probiotics, identifying components of the microbiota-gut-brain axis might result in new approaches to treat neurodegenerative and neuroinflammatory diseases.

Post-stroke gut dysbiosis shapes immunity and metabolism

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Stroke is characterized by the recruitment of circulating immune cells to the injured brain. We have shown that stroke induces gut dysbiosis as well as the migration of intestinal immune cells from the gut to the brain where they contribute to neuroinflammation and secondary brain injury. Thus, understanding how immune cells are modulated in the gut has strong therapeutic implications.

Metabolites derived from gut microbiota regulate the intestinal immune response. In addition to serving as a nutrient enhancer, tryptophan metabolites play crucial roles in the regulation of the intestinal immune tolerance. Importantly, tryptophan is the common precursor of ligands of the transcription factor aryl hydrocarbon receptor (AhR). AhR is highly expressed in immune cells and regulate their function by inducing T cell polarization directly or via dendritic cells.

Here, I will focus on how the tryptophan/AhR metabolism regulates peripheral immune response in a context of stroke. Findings derived from this study may allow for the identification of innovative therapies based on microbiota-metabolites to prevent or alleviate intestinal inflammation and stroke progression.

The Effect of Age-Related Dysbiosis on Post-Stroke Translocation of Gut Bacteria

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Question: Chronic systemic inflammation contributes to the pathogenesis of many age-related diseases, including stroke. Alterations in the gut microbiota, or dysbiosis, may contribute to age-related inflammation and increase rates of post-stroke infection, a leading cause of stroke-associated mortality.

Methods: We investigated the effects of age and the role of bacterial translocation from the gut in young (8-12 weeks) and aged (18-20 months) C57Bl/6 male mice following transient middle cerebral artery occlusion (MCAO) or sham surgery. Gut permeability was examined and peripheral organs were assessed for the presence of gut-derived bacteria following stroke. Furthermore, sickness parameters and components of innate and adaptive immunity were examined. In subsequent experiments, we tested the hypothesis that a youthful microbiota, when established in aged mice, would improve outcomes following ischemic stroke. Young and aged male mice had either a young or an aged microbiota established by fecal transplant gavage (FTG). Mice were subjected to ischemic stroke (MCAO) or sham surgery. During the subsequent weeks, mice underwent behavioral testing and fecal samples were collected for 16S ribosomal RNA analysis of bacterial content.

Results: We found that while stroke induced gut permeability and bacterial translocation in both young and aged mice, only young mice were able to resolve infection. Bacterial species seeding peripheral organs also differed between young (Escherichia) and aged (Enterobacter) mice. Consequently, aged mice developed a septic response marked by persistent and exacerbated hypothermia, weight loss, and immune dysfunction compared to young mice following stroke. The microbiota is altered after experimental stroke in young mice and resembles the biome of uninjured aged mice. In aged mice, the ratio of Firmicutes to Bacteroidetes (F:B), two main bacterial phyla in gut microbiota, increased ~9-fold ($p < 0.001$) compared to young. This increased F:B ratio in aged mice is indicative of dysbiosis. Altering the microbiota in young by fecal gavage to resemble that of aged mice (~6-fold increase in F:B ratio, $p < 0.001$) increased mortality following MCAO, decreased performance in behavioral testing, and increased systemic cytokine levels. Conversely, altering the microbiota in aged to resemble that of young (~9-fold decrease in

F:B ratio, $p < 0.001$) increased survival and improved recovery following MCAO. New unpublished data showing that altering the biome, even several days after stroke, continues to have therapeutic benefit in aged mice.

Conclusion: Aged mice have higher systemic inflammation and gastrointestinal dysfunction, which is worse after stroke. In addition, the microbiome in aged animals is dysbiotic, leading to systemic seeding of deleterious bacteria and poorer outcomes after brain injury. Manipulating the components of the microbiome is feasible and improves recovery, even when altered several days after stroke.

Endovascular approach to the treatment of stroke: beyond thrombectomy

Inflammation and Stroke

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Stroke is the primary cause of long-term disability and the third leading cause of death in industrialized countries. In Germany, the annual incidence of stroke is above 250,000. The lifetime risk of experiencing a stroke ranges from 8% to 10% and continues to rise due to demographic changes. New avenues of basic research with high translation potential are desperately needed in order to develop effective therapeutic strategies. While the development of local inflammatory processes in the ischemic brain is a known phenomenon, precisely how these immune processes are linked to secondary expansion of the infarct area and the role of the immune system in post-stroke regeneration remain poorly understood. We have recently described some key elements of the inflammatory cascade such as ATP, IL-17, or MMP-9 which might serve as good candidates for therapeutic interventions. I will talk about the exact mechanisms and potential future therapies.

Precise targeting of therapeutics to stroke under interventional MRI

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The concept of stem cell-based therapy for stroke is now few decades old but the progress is far from satisfactory. Large body of preclinical studies indicate improvements after cell transplantation but clinical trials so far fail to show functional benefit or improvements are too small to justify large scale treatment. One unsolved issue that is likely contributing to suboptimal treatment outcomes is related to suboptimal cell delivery to stroke lesion. Several routes of cell delivery have been considered including direct stereotaxic injection, intraventricular, intraarterial or intravenous route with the last one being most extensively used. While intravenous route is convenient and safe, only marginal amount of cells reach the brain. Intraparenchymal and intraventricular injections force cell placement within the brain but extensive migration is needed for the cells to reach entire lesion. Intraarterial route on the other hand offers unique opportunity for highly selective delivery of stem cells to the brain and their uniform distribution throughout the lesion.

We observed that intraarterially infused cells indeed are capable of homing to the brain but their biodistribution is highly variable and affected by multiple factors such as size and type of cells, infusion parameters or streaming effect. For these reasons we have developed interventional MRI cell tracking as a strategy for highly predictable precise and reproducible delivery of stem cells to desired brain structures. With increasing use of mechanical

thrombectomy as a treatment strategy for stroke intraarterial route for delivery of stem cells is even more appealing as cells could potentially be infused as adjuvant therapy during the same endovascular procedure.

Magnetic Particle Imaging for Neurological Applications

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Magnetic particle imaging (MPI) is a new background-free tomographic imaging method which directly detects superparamagnetic iron oxide-based contrast agents (SPIOs).

Compared to other conventional imaging methods, MPI combines a superior temporal resolution (21.5ms) with a good spatial resolution (0.4mm) allowing 3D real-time assessment of vasculature and perfusion without X-rays or nephrotoxic contrast agents. In addition, MPI scanners can be built as prehospital mobile devices, which require less complex infrastructure than computed tomography (CT) and magnetic resonance imaging (MRI). In this talk, we show that MPI can be used for the diagnosis of acute pathologies like ischemic stroke. Cerebral ischemia was induced by inserting of a microfilament in the internal carotid artery in C57BL/6 mice, thereby blocking the blood flow into the medial cerebral artery. After the injection of a contrast agent specifically tailored for MPI, cerebral perfusion and vascular anatomy were assessed by the MPI scanner within seconds. For the first time, we showed that MPI could be used as a diagnostic tool for relevant diseases in vivo, such as an ischemic stroke. Due to its shorter image acquisition times and increased temporal resolution compared to that of MRI or CT, we expect that MPI offers the potential to improve stroke imaging and treatment.

Additionally, we give an outlook how MPI may revolutionize not only stroke imaging but also stroke treatment, as the magnetic fields of the MPI can be used for catheter guidance and targeted drug delivery.

Endovascular model of stroke in swine

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Stroke is a significant burden to our society and due to population aging and sedentary lifestyle its incidence is expected to grow. Tissue plasminogen activator (tPA) is the only FDA-approved drug for treatment of acute stroke but its efficacy is rather low and side effects are frequent. Rodent models play an important role in studying stroke and developing therapies; however, relying solely on rodents proved inadequate as practically all clinical trials, that were based solely on rodent data failed. STAIR and STEPS advisory bodies recommend testing experimental therapies of stroke in large, gyrencephalic animals prior to first-in-man studies. Endovascular approaches are widely used in clinic because of their non-invasive nature. We explored utility of this method to induce ischemia in pigs with high clinical relevance. Percutaneous puncture was made to insert introducer (5F,Prelude MeritMedical) into femoral artery followed by guiding catheter using C-arm guidance. Microcatheter was placed in ascending pharyngeal artery (APA) proximally to the *rete mirabile*. Next phase of the experiment was made under guidance of

real time MRI using 3T scanner (Magnetom Trio, Siemens). To induce ischemia we explored utility of thrombin infused intra-arterially into pharyngeal ascendant artery just proximal to rete. MRI protocol included dynamic GE-EPI for assessment of trans-catheter cerebral perfusion, GE-EPI for monitoring thrombin-mediated blood clotting as well as SWI, diffusion, T2w and T1w with contrast. Images were analyzed using AMIRA 6.4 to visualize microcatheter perfusion. Contrast-enhanced perfusion MR scans showed brain territory supplied by the catheter infusion at three time points – baseline, thrombin administration, 30 min after stroke induction. Brain perfusion was dynamically quantified, what showed significant reduction in perfusion in ipsilateral hemisphere after thrombin administration (8,754.4 mm3). T2w scan 17 hours post stroke confirmed ischemia. Interestingly contrast enhanced MRI indicated that BBB was intact within initial 17 hours, but BBB breach has been observed one month after surgery. Our study demonstrated feasibility of endovascular stroke model in swine. We were able to observe in real-time formation and evolution of stroke lesion. Our model produced infarct volume covering MCA territory. Overall, we established novel model of ischemic stroke in pigs with high clinical relevance.

Microcirculation and capillary function

Capillary Function: Implications for tissue oxygenation in the normal and diseased brain

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We are used to think, that brain oxygenation depends solely on its regional cerebral blood flow (CBF). This assumption is only true, however, if blood is uniformly distributed across tissue capillaries. The capillary distribution of blood flows can be characterized by the standard deviation of blood"s capillary transit times, so-called capillary transit-time heterogeneity (CTH). Notably, non-zero CTH reduces oxygen extraction efficacy in tissue, and disturbed capillary function (high CTH) may be a source of hypoxia in brain tissue, even when though its blood supply is inconspicuous (non-ischemic). The talk will describe how tissue oxygenation depends on CBF and CTH combined, how CTH can be measured, and how it behaves in healthy and diseased brain.

Improving microcirculatory reperfusion can promote stroke outcome after recanalization therapies

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Recent research on microcirculation has led to an unprecedented progress. This progress has been complemented with discovery of important roles of microcirculation in brain diseases. Evidence from clinical trials consistently shows that recanalization does not always lead to reperfusion of the ischemic tissue in stroke patients treated with tPA or interventional methods. A recent study that specifically evaluated the relationship between reperfusion and recanalization reported that reperfusion could not be attained in 57% of the recanalized patients receiving tPA within 4.5 hours. If microcirculatory blood flow cannot be sufficiently reinstituted despite complete recanalization as observed in coronary circulation, it may be one of the factors reducing efficacy of thrombolysis in stroke. Experimental studies suggest that the damage induced by ischemia to cerebral microvessels limits reperfusion (no-reflow) after recanalization and plays a critical role in determining tissue survival. MR studies specifically measuring capillary transient time in stroke patients support these experimental findings and underscore the importance of capillary transient time heterogeneity in tissue oxygenation beyond the patency of upstream vessels. Recent experimental studies with adenosine nanoparticles or inhaled NO bring about the exciting possibility that benefit of reperfusion therapies can be enhanced by restoring microcirculatory function. For instance, improving reperfusion alone (e.g. with blood-brain barrier impermeable agents) significantly reduces parenchymal oxygen radical formation. In light of these recent developments, we think that therapies targeting microcirculation is the next step to improve the success of recanalization therapies for ischemic stroke. Combination of parenchymal neuroprotection with improved microvascular reperfusion is likely to be an ideal approach. Recent interventional trials clearly show that a therapeutic window beyond 6 hours exists if the tissue is supported by residual blood flow at penumbral

levels, which creates hopes that current achievement of recanalization therapies can significantly be improved by combining microvascular and parenchymal protection.

Compromised microvascular oxygen delivery increases brain tissue vulnerability with age

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Much is still unknown about the changes in brain tissue pO_2 with age. Using a detailed investigation of the age-related changes in cerebral tissue oxygenation in the barrel cortex of healthy, awake aged mice, we measure decreased arteriolar and tissue pO_2 with age using two-photon lifetime phosphorescence microscopy. We further uncovered the presence of hypoxic micro-pockets in the cortex of awake old mice. Our data suggests that from young to middle-age, a well-regulated capillary oxygen supply maintains the oxygen availability in cerebral tissue, despite decreased tissue pO_2 next to arterioles. After middle-age, due to decreased hematocrit, reduced capillary density and higher capillary transit time heterogeneity, the capillary network fails to compensate for larger decreases in arterial pO_2.

Conditioning Medicine: Basic Mechanisms and clinical potential

Immune response and tolerance

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The innate immune system plays a dualistic role in the evolution of ischemic brain damage and has also been implicated in ischemic tolerance produced by different conditioning stimuli. It is well known that activation of the innate immune system is an important component of the inflammatory response and utilizes toll-like receptors.

On one hand, we and others have demonstrated that TLR4 signalling is involved in brain tolerance as shown by the difference in the percentage of neuroprotection produced by ischemic preconditioning (IPC) between TLR4KO animals and mice expressing normally TLR4. The higher expression of TNF-**a**, iNOS and cyclooxygenase- 2 and NF-**k**B activation in mice expressing TLR4 is likely to participate in this endogenous neuroprotective effect. It has been also demonstrated that the application of recombinant high-mobility group box 1 (rHMGB1) or lipopolysaccharide (LPS), as TLR4 ligands (DAMPs or PAMPs respectively), as preconditioning stimuli prior to cerebral ischemia reduced the subsequent cerebral damage and improved neurological function. Ischemic tolerance has been also demonstrated in human clinical practice: less severe strokes have been described in patients with prior ipsilateral transient ischemic attacks within a short period of time.

On the other hand, we have also shown that TLR4 deficiency significantly attenuates ischemic brain damage and hemorrhagic transformation, at least in part, by decreasing brain inflammatory response. The brain responds to ischemic injury with an acute and prolonged inflammatory process, characterized by rapid activation of microglial resident cells but also promoting cell infiltration into the ischemic tissue of various types of inflammatory cells such as neutrophils, monocyte/macrophages and different subtypes of T cells among others. Interestingly, TLR4 expression in peripheral immune cells has been previously demonstrated to play a role in mediating ischemic damage in experimental stroke models. In particular, TLR4 expression on neutrophils has been also correlated with poor functional outcome after stroke and intracerebral hemorrhage. Our group previously demonstrated that, after stroke, the population of infiltrated neutrophils in the brain is heterogeneous, including a population of alternative

neutrophils (N2) that express M2 phenotype markers. In this context, TLR4 modulates neutrophil polarization since TLR4 deficiency increases the levels of alternative neutrophils (N2) which might be involved in the resolution of inflammation and in the neuroprotective effect observed after stroke.

The dual role of TLR4 in the processes of cerebral ischemia as both neuroprotective (ischemic tolerance) and damage mediator (acute ischemia) will be discussed.

Dieting to condition the brain: caloric restriction and the preservation of ischemic brain

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The beneficial effects of dietary restrictions have been observed for decades, including in the context of aging. Protection against ischemic injury can also be conferred by dietary restriction, but the underlying mechanisms are not well understood. We have recently found that dietary restriction prior to cerebral ischemia protects both grey and white matter in the brain. Furthermore, we identified that adiponectin is released systemically in response to dietary restriction, and that manipulation of adiponectin levels affected the extent of protection afforded to the ischemic brain. In addition to these observations, other contributors to the conditioned state elicited by dietary restriction (including glucose levels and sirtuins) may also work toward a unique state of conditioning. The discussion of these potential mechanisms contributing to the conditioned brain by dietary restriction will be discussed, as well as inclusion of white matter in the assessment of ischemic tolerance.

Long-term window of ischemic tolerance

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Our lab recently reported that resveratrol preconditioning affords tolerance against a focal cerebral ischemic insult in mice that can last for at least 14 days in vivo making it the longest window of ischemic tolerance discovered to date by a single administration of a pharmacological agent. The mechanism behind this novel extended window of ischemic tolerance remains elusive. In this presentation I will discuss potential mechanisms that could explain this novel extended window of ischemic tolerance in the context of previously identified windows and the known mechanisms behind them. I will also draw parallels from the fields of hibernation and hypoxia-tolerance, which are chronic adaptations to severe conditions of hypoxia and ischemia known to be mediated by a form of metabolic depression.

Anesthetic Postconditioning in Acute Brain Injuries —Anesthetics beyond Anesthesia

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Acute brain injuries like ischemic stroke, traumatic brain injury (TBI) and intracerebral hemorrhage (ICH), if not treated properly, may result in sensory, motor and cognitive function impairments, seriously affecting the life quality of patients. Although considerable progress has been made in invasive interventions and surgical treatments of these diseases, including endovascular thrombectomy, decompressive craniotomy and et al, the management of acute brain injuries still need further attention due to their devastating impacts on neurological outcomes. Anesthetic postconditioning is emerging as a promising therapeutic strategy with advantages of easy accessibility, high controllability and its great effect on balancing metabolic demand, glutamatergic and GABAergic tone, controlling cortical spreading depression, neuroinflammation and ROS generation in the injured brain, which are all important pathological features of acute brain injuries. The advances in anesthesiology, including the development of new anesthetic agents and the innovation of anesthetic drug delivery system, could contribute to the clinical

translation of anesthetic postconditioning in the early management of acute brain injuries in an effort to further improve the neurological outcomes. Here in this talk, clinical aspects for the application of anesthetic postconditioning in acute brain injuries will be discussed, including patient management, postconditioning paradigms, and the design of clinical trials on this topic.

Targeting Astrocytes for CNS disease

Targeting aquaporin-4 in astrocyte to reduce edema

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Edema is a hallmark of several brain disorders including stroke, traumatic brain injury. Edema is water accumulation resulting from dysregulation of osmotic homeostasis resulting to the brain swelling. Currently, there are only limited treatments to prevent edema formation in various brain disorders. Therefore aquaporin 4, a brain water channel, has become a focus of interest for therapeutic approaches targeting edema. At present, there are no pharmacological specific tools to block AQP4. The role of AQP4 in edema after brain injury remains unclear with conflicting results from various studies. Presence of AQP4 has been proposed to be beneficial or deleterious depending of the injury models, the tissue location. Interestingly, absence of AQP4 confers an overall beneficial role at long-term with improved neuronal survival and reduced neuroinflammation after ischemic stroke, but without a direct effect on edema formation. These results strongly suggest that AQP4 has other roles and could be involved in neuroinflammation with facilitation of the astrocyte migration in glial scar formation. The conflicting results and various roles of the AQP4 will be reviewed during the presentation.

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Astrocyte-specific gene transfer of Insulin-like Growth Factor (IGF)-1 in middle aged female rats improves stroke outcomes

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Ischemia-induced brain infarction is more severe in middle-aged female rats than younger female rats, and this is associated with an age-related loss of circulating and parenchymal brain levels of insulin like growth factor-1 (IGF-1). IGF-1 synthesis is decreased in middle-aged astrocytes, a cell type that is critical for post stroke recovery. In this study, we tested the hypothesis that astrocyte-specific IGF-1 gene transfer to middle-aged females will improve stroke outcomes. Middle-aged (10-12 mo old, acyclic) female rats were injected with adeno-associated virus serotype 5 (rAAV5) packaged with the coding sequence of the hIGF-1 gene downstream of an astrocyte-specific promoter (GFAP) into the striatum and cortex. The rAAV-control consisted of an identical shuttle vector construct without the hIGF-1 gene. Six-eight weeks later, animals underwent 90-minute transient middle cerebral artery occlusion (MCAo) via intraluminal suture. At 1d post stroke, flow cytometry was used to determine the type and extent of peripheral immune cells trafficked into the brain. In parallel studies, animals were tested for performance on sensory motor tasks at 2 and 5 days after MCAo. rAAV-mediated IGF-1 expression was confirmed in astrocytes with RT-PCR. Flow cytometry analysis of immune cells in the brain at 24h post stroke found that proportion of Treg cells was greater in animals with rAAV-IGF-1 as compared to rAAV-controls. Additionally, while there was no difference in the proportion of M2 microglia, rAAV-IGF-1 enhanced M2 infiltrating macrophages. At 2d and 5d post stroke, stroke induced sensory motor impairment was reduced in animals with rAAV-IGF-1 as compared to rAAV-controls. The present study indicates that targeted enhancement of IGF-1 in astrocytes of middle-aged female

improved stroke-induced behavioral impairment concomitant with recruitment of anti-inflammatory cell types to the ischemic brain.

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Targeting Astrocyte Activation with Interleukin-1 alpha is Therapeutic in Ischemic Stroke

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A primary event in stroke pathogenesis is the development of local and peripheral inflammatory responses known to contribute to poor outcome and impaired functional recovery. This inflammatory cascade includes the activation of astrocytes, microglia, and peripheral immune cells that infiltrate the brain causing further injury. Post-stroke inflammation is regulated in part by the pro-inflammatory "master" cytokine interleukin-1, and while the role of IL-1b in stroke pathogenesis is well studied, the role of IL-1a remains largely unknown but may be significant as IL-1a brain levels are profoundly affected by stroke. Importantly, clinical studies blocking IL-1 mediated inflammation (with IL-1RA) have failed to produce significant benefit. Because of this, we hypothesized that IL-1a could impart positive, rather than negative, effects in the brain following stroke.

Hypothesis: IL-1a could represent a novel neuroprotective therapeutic target in ischemic stroke that works by dampening brain inflammation, including astrocyte activation. We also hypothesize that effects of peripherally administered IL-1a on pulse distension and core temperature might be minimized or eliminated by intraarterial administration. Finally, we hypothesize that IL-1a could be working through generation and proteolytic cleavage of the extracellular matrix proteoglycan perlecan into its neuroprotective and pro-astrocyte DV protein subunit.

Methods: Young wild-type and perlecan deficient (pln -/-) male mice were subjected to middle cerebral artery occlusion for 1 hour. Animals were treated with IL-1a via intravenous (IV) or intraarterial (IA) injection immediately upon reperfusion during which pulse distension and core temperature were recorded. Infarct volume was quantified using cresyl violet staining, astrocyte and microglial activation was assessed through GFAP and CD11b immunostains, respectively, and apoptotic cell death was evaluated via TUNEL staining. Behavioral deficit and recovery was scored using an 11-point score.

Results: Wild-type mice treated via IA IL-1a injection had reduced effects on pulse distension and temperature and smaller infarct volumes than those receiving IV injection. Both IV and IA treated mice incurred less functional deficit and less cerebral inflammation (astrocyte activation and microglial activation) than vehicle controls. However, pln -/- mice did not gain the same neuroprotection after IL-1a treatment.

Conclusion: Administered IL-1a is neuroprotective in experimental stroke with minimal systemic side effects (IA), and requires the presence of perlecan for its therapeutic effect. IL-1a may represent a novel stroke therapy.