Poster Session 6: Miscellaneous topics

P I - 6-1
An emerging role for Hsp27 in VCID.
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Background: Hyperhomocysteinemia (HHcy) is a risk factor for vascular cognitive impairment and dementia (VCID), as well as Alzheimer’s disease. The mechanism by which HHcy promotes VCID or AD remains unknown. Using the HHcy mouse model of VCID, we found the earliest detectable event in the brain is a robust neuroinflammatory response. This is followed by neurovascular astrocyte disruptions, cerebral hypoperfusion, microhemorrhages, white matter degeneration, and cognitive impairment.

To gain mechanistic insights into the signaling pathways by which HHcy induces this sequelae of events, we focused on heat shock protein 27 (Hsp27). Hsp27 binds protein-folding intermediates and prevents their aggregation without directly refolding them itself. Aberrant phosphorylation of Hsp27 has been reported in a variety of cancers and neurodegenerative diseases. Given that Hsp27 is shown to be involved in cerebrovascular dysfunction in stroke models, and is known to signal through the p38 MAPK signaling pathway, a critical driver of the proinflammatory response, we hypothesized Hsp27 is an early mediator of HHcy-induced neuroinflammation, and therefore, the downstream events that occur as a result.

Methods: Wildtype and Hsp27-/- mice were subject to a HHcy-inducing diet for 14 weeks. Tissue from the left hemisphere was histologically examined for microglial activation, the astrocytic end-foot integrity, and microhemorrhages. Tissue from the right hemisphere was used to evaluate the neuroinflammatory state using qRT-PCR. Western blot and MSD were used to confirm some of the identified proteins.

Results: Our wildtype-HHcy model displayed significant pro-inflammatory responses and astrocytic end-foot disruptions, as well as significant microhemorrhage induction. Significantly, we found that there was no induction of the pro-inflammatory phenotype in the Hsp27-/- mice subjected to the HHcy-inducing diet for 14 weeks. We also found a reduction in the microhemorrhage incidence in the Hsp27-/- mice, as well as improved survival of the mice, indicating that they were resistant to the HHcy diet.

Conclusions: Hsp27 appears to be an early essential mediator of HHcy-induced pathology. Deletion of Hsp27 provides protection from diet-induced attrition, neuroinflammation, and cerebrovascular events. This suggests that Hsp27 may be an attractive therapeutic target for the treatment of VCID.

P I - 6-2
Investigating metabolic regulation of the cerebrovasculature.
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Vascular dementia (VaD) is the second leading cause of dementia and, although accounting for up to 30% of all dementia cases, it is poorly understood and under-researched. Cerebral ischemia is a prominent pathological feature of VaD that is caused by occlusion of large blood vessels supplying the brain and/or degeneration of small intracerebral blood vessels. Inflammation, normally a protective response by the host to infection or injury, has been shown to exacerbate cerebral ischemia and is associated with the development of dementia. In agreement, clinical observations and preclinical models of VaD have indicated vascular dysfunction and inflammation as key drivers of disease pathology.
Our understanding of how reprogramming of metabolic pathways alter inflammatory cell function has grown considerably in recent years, and has provided insight into pathological mechanisms of disease. In the context of VaD, viewing inflammation from a metabolic perspective may aid in understanding how endothelial cells, which represent the interface between vasculature and brain, become dysfunctional and contribute to disease pathology. We aim to dissect the metabolic pathways that mediate inflammation in the cerebrovasculature with the ultimate aim of identifying potential metabolism-centric treatment strategies for VaD.

Using *in vitro* model systems we are investigating the metabolic regulation of the inflammatory phenotype of brain endothelial cells in response to VaD-relevant stimuli, such as oxygen-glucose deprivation and disturbed flow. Ultimately, understanding the complex interplay between metabolism and inflammation at the cerebrovascular interface may provide a greater insight to the molecular mechanisms of disease progression in VaD.

**P I - 6-4**

Posttranslational-modifications of oligomeric amyloid beta (AβO) trigger synaptic dysfunction by their own mechanisms in Alzheimer’s disease (AD)

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Numerous researches reported that soluble oligomeric amyloid beta (AβO) disrupts synaptic plasticity and memory at an early stage of Alzheimer’s disease (AD). Various posttranslational-modified AβO have been identified, and the most abundant types are N-terminally truncated forms. To address the question whether modifications of AβO can trigger synaptic dysfunction on their own, we show that 1) the D1/D5R agonist protects long-term potentiation (LTP) of hippocampal CA1 acute slices from the deleterious action of oligomeric amyloid beta 1-42 (Aβ1-42); 2) and the inhibition of Src-family tyrosine kinases completely abolishes the protective effects of D1R/D5R stimulation. Moreover, we detect that 1) a prominent isoform, pyroglutamated Aβ3 (pE)-42, triggers synaptic dysfunction to a similar extent like Aβ1-42 but by clearly different mechanisms; 2) in contrast to Aβ1-42, Aβ3(pE)-42 induced synaptic dysfunction is not related to NMDAR signalling but links to glial releasing of the pro-inflammatory cytokine tumor necrosis factor α (TNFα), and Aβ3(pE)-42 induced impairment of synaptic plasticity cannot be rescued by D1R/D5R-agonists. Collectively, our data point to a scenario where neuroinflammatory processes together with direct synaptotoxic effects are caused by posttranslational modification of soluble oligomeric Aβ and contribute synergistically to the onset of synaptic dysfunction in AD.
MarkVCID: Vascular contributions to cognitive impairment and dementia biomarkers development and validation

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The World Health Organization reports that 47.5 million people are affected by dementia worldwide. The burden of illness due to dementia approaches crisis proportions. Evidence from epidemiology and pathology studies indicates that damage to the vascular system is associated with an increased risk of many types of dementia and neurodegeneration. The science of vascular contributions to cognitive impairment and dementia (VCID) integrates diverse aspects of biology and incorporates the roles of multiple cell types that support the function of neural tissue including lipoprotein metabolism. The MarkVCID consortium, established by the NINDS/NIH in 2016 via 2 RFAs, develops and rigorously validates candidate biomarkers for small vessel VCID. The goal of the consortium is to deliver high-quality VCID biomarkers ready for use in large scale clinical trials. During phase I (years 1-2; year 2 is in presently in progress), biomarker development projects (sites), with support from the Coordinating Center, have established the consortium, including consortium agreements, best practices and standardized protocols, and have further developed candidate biomarkers. During phase II (years 3-5) the consortium sites will independently perform cross-site validation studies to further evaluate and develop biomarker candidates to readiness for large scale multi-site clinical validation studies and, if successful, for use in interventional clinical trials. To facilitate the transition from phase I to phase II, the seven sites recently nominated more than a dozen biomarker paradigms, including both fluid biomarkers and imaging biomarkers, designed to diagnose, determine risk for, monitor, and prognose small vessel VCID. After a selection process including input from the Coordinating Center on behalf of the MarkVCID consortium, an External Advisory Committee, and NINDS leadership, independent cross-site testing and validation of the most promising small vessel VCID biomarkers is scheduled to start in July of 2018. Here we will report the outcome of the selection process and progress on multi-site validation of selected candidate small vessel VCID biomarker paradigms.
Neuron and astrocyte metabolic responses to the proteasome inhibitor in Alzheimer’s disease

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The changes in energy metabolism are the earliest events that occur in the Alzheimer disease (AD) affected brain. Mitochondria produce most of the cell energy and play a central role in aging and neurodegeneration. Different nerve cells have various metabolic profiles allowing them to play complementary functions in supporting brain metabolism. Neurons primarily generate energy through the tricarboxylic acid cycle and oxidative phosphorylation, while astrocytes produce ATP mainly through glycolysis. Mitochondria are thought to be excellent optical targets because they exhibit strong autofluorescence associated with two cofactors involved in energy metabolism – NAD(P)H and FAD. Fluorescence lifetime imaging microscopy is a powerful technique for studying cell metabolism via endogenous fluorescence of metabolic cofactors. The ubiquitin-proteasome system is known to take part in degradation of proteins involved in various cellular processes like metabolism, inflammation, apoptosis and synaptic plasticity. Nevertheless, the role of proteasome in regulation of nerve cell metabolism in AD is not well understood yet.

The aim of this investigation was to study the metabolic responses of neurons and astrocytes under the proteasome inhibitor treatment using confocal fluorescence microscopy and fluorescence lifetime imaging (FLIM). Neurons and astrocytes were isolated from freshly dissected embryonic mouse hippocampus of 5XFAD mouse model of Alzheimer’s disease. For metabolism study, primary hippocampal culture was incubated with the proteasome inhibitor MG132 before fluorescence imaging. Detection of fluorescence intensity and fluorescence lifetime of the metabolic cofactors (NAD(P)H) and FAD was performed using the FLIM system (Becker&Hickl GmbH., Germany) on LSM 710 microscope (Carl Zeiss, Germany).

Analysis of neuron metabolic response to the proteasome inhibitor in AD showed the increase in the redox ratio FAD/NAD(P)H in 2 hours after MG132 exposure. At the same time, FAD/NAD(P)H ratio did not change significantly in astrocytes after the proteasome inhibitor treatment. NAD(P)H fluorescence lifetime imaging revealed the decrease in relative amplitude of free form of NAD(P)H in both hippocampal neurons and astrocytes upon MG132 treatment. It can be associated with oxidative metabolic activity or oxidizing conditions. Mitochondrial damage in neurons in AD may lead to reduced efficiency and a compensatory increase in oxidative phosphorylation.

Therefore, a shift in energy production can potentially be used as cellular metabolic metrics to estimate the condition of AD at the cellular level. Moreover, modulation of the proteasome activity may be considered as a new strategy in AD treatment.

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Bioluminescence imaging reveals neurogenesis and neuroblast migration induced by osteopontin in the mouse brain after experimental ischemia

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Osteopontin (OPN) is an endogenous pleiotropic phosphoglycoprotein that is upregulated in the brain after cerebral ischemia. OPN supports migration, survival, and proliferation of neural stem cells (NSC) in primary cell culture, as well as their differentiation into neurons, as reported previously by our group. The aim of this study was to analyze the effects of OPN on neuroblasts in vivo in the context of cerebral ischemia.

OPN was injected intracerebroventricularly in transgenic mice expressing luciferase (luc) under the control of the neuroblast-specific doublecortin- (DCX-)promotor, allowing visualization of neuroblasts in vivo using bioluminescence imaging (BLI). Control mice were injected with vehicle-buffer. For ex vivo immunohistochemical assessment of the effects of OPN after ischemia, additional mice were subjected to photothrombosis, and injected with either OPN or vehicle.

BLI in vivo showed that OPN enhanced the migration of neuroblasts both in the healthy brain as well as after ischemia. In the ischemia condition, OPN treatment also recruited neural progenitors from the contralateral hemisphere. Additionally, neural progenitor numbers were increased following OPN-treatment, with the maximum effect on the second day after OPN-injection into the healthy brain, and 14 days after OPN-injection following ischemia.

Our results strongly suggest that OPN constitutes a promising treatment for the targeted activation of neurogenesis in ischemic stroke.

Prominent vessels on quantitative susceptibility maps indicate microvascular pathology after experimental cerebral ischemia and reperfusion

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Question: Prominent vessels in the brain of patients with ischemic stroke have been observed on susceptibility weighted images and quantitative susceptibility maps (QSM). However, the occurrence of prominent vessels after restoration of reperfusion has so far not been evaluated. The goal of the current study was to perform QSM in the middle cerebral artery occlusion (MCAO) model of cerebral ischemia and to quantitatively assess the...
occurrence of prominent vessels. Moreover, immunohistochemistry was used to assess underlying vessel pathology in brain sections.

**Methods:** Mice underwent 1h of MCAO followed by reperfusion using the intraluminal filament technique. A Bruker PharmaScan 47/16 operating at 200 MHz and equipped with a cryogenic transmit-receive coil was used for MRI. During acquisition mice were spontaneously breathing under isoflurane anesthesia (1.5%). A 3D multi-echo gradient recalled echo sequence was applied using a FOV=25.6mm x25.6mm x8mm and an acquisition matrix=256x256x80, resulting in an effectively isotropic spatial resolution of 100μm x100μm x100μm. Four echoes were recorded (TE1-4=4.5/10.5/16.5/22.5ms) with TR =100ms, flip angle =15°. Data was postprocessed for the generation of susceptibility maps and volume-of-interest (VOI) analysis was performed. Brain sections were prepared and stained with anti-mouse collagen IV.

**Results:** Prominent vessels with high magnetic susceptibility were seen on magnetic susceptibility maps of the ischemic hemisphere on all time points. Prominent vessels appeared larger in diameter than comparable vessels on the contralateral side. Furthermore, an increased number of prominent vessels were found in the ischemic hemisphere of mice imaged at 12h, 24h and 48h after reperfusion compared to mice imaged at 2h, 4h and 6h after reperfusion. Significantly higher differences in magnetic susceptibility were found in prominent vessels of the ischemic ipsilateral side compared to the contralateral hemisphere at 2h and 4h after reperfusion. Immunohistological examination demonstrated appearance of dilated larger vessels and capillaries with swollen endothelial cells in combination with narrowing of the vessel lumen.

**Conclusion:** Microvascular pathology hampers reperfusion of ischemic tissue and promotes secondary tissue injury. Thus, prominent vessels are an important indicator of underlying microvascular pathology and may be pivotal for diagnosis and therapeutic decision making in stroke patients.

**P I - 6-9**

**A novel automated tool for analysis of microglial morphology**

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**Questions:** Microglia are the resident immune cells of the brain and react quickly to changes in their environment with transcriptional regulation and morphological changes. An ischemic stroke induces local inflammatory responses encompassing microglial activation, which is reflected in gradual morphological changes from a highly ramified into a less ramified or amoeboid cell shape. For this reason, morphological changes of microglia are widely utilized to quantify microglial activation and studying their role in virtually all brain diseases. However, the currently available methods, which are mainly based on manual ratings of immunofluorescent microscopic images, are often inaccurate, rater biased and highly time consuming.

**Methods:** The study was conducted in 10 to 12 weeks old male mice, operated for distal middle cerebral artery occlusion for stroke induction. We stained brain sections immunohistochemically for Iba1+ microglia and acquired confocal Z-stacks. The morphology of microglia in an exploratory data-set was first analysed manually using Sholl- and circularity analysis. We created a fully automated algorithm for the analysis of microglial morphology. The main steps of the algorithm comprise quality control and pre-processing, as well as segmentation of microglia from the background and into cell compartments. The spatial structure of microglia was represented by skeletonization. This information was used to extract up to 59 morphological features. Dimensionality reduction of these features by principal component analysis (PCA) allowed to generate a compound score for microglial shape analysis. We validated the functionality of the tool on a second independent data-set and performed correlation analysis on both data-sets. Finally, we tested for concordance of results between the automated analysis tool and the conventional manual analyses.
Results: Manual analysis revealed morphological differences between microglia in the peri-infarct and contralateral brain areas. We detected similar morphological differences in the same data-set with our novel automated analysis tool and verified the functionality of the algorithm in the validation data-set. The compound score, resulting from the PCA, provided a sensitive feature for microglial morphology and was able to detect a significant difference between microglia in the peri-infarct and contralateral area. Further, it was sensitive to even detect subtle morphological differences within the peri-infarct area. The results from both manual and automated analysis showed positive correlation.

Conclusions: Our novel tool for microglia morphology analysis presents a fully automated, reliable and objective method to analyse microglia morphology. Due to the high discrimination performance, we expect that the sensitivity of the tool is highly suitable to distinguish morphological changes of microglia in a broad range of brain diseases.

Factors affecting the biodistribution of glial progenitors transplanted in the piglet cerebral ventricular system using a 3D printed model

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Question: Neonatal hypoxia-ischemia continues to be a significant problem, despite therapeutic advances of hypothermia. We have previously shown that intra-ventricular delivery of human glial progenitors (GPs) at the neonatal stage is capable of replacing host abnormal glia cells, rescuing the life span of dysmyelinated mice. However, the small size of the murine brain does not allow proper investigation of the transplantation challenges related to high-volume ventricles and long transport distances for stem cells as encountered in the human brain. Our long-term goal is to study the potential benefits of complete glia replacement in a large animal (piglet) model of neonatal hypoxia-ischemia. Because the cerebral ventricles would be an attractive gateway to introduce cells to vast brain areas across the entire neuroaxis, we used bioluminescence (BLI) and real-time MRI to investigate the potential variables that could maximize the biodistribution of injected glial progenitors within the ventricular system while minimizing their outflow to the intrathecal space.

Methods: 3D model preparation: A 3D model of the piglet cerebral ventricles was rendered based on T2-weighted MR images (11.7T Bruker Biospin) and post-mortem brain dissection with subsequent 3D printing (Stratasys). Stem cell infusion: SPIO-labeled (Molday ION Rhodamine B, Biopal) and unlabeled Luc(GP) were infused into our 3D printed artificial ventricles using a port mimicking a trajectory typically used for shunt placement, which is a frequent pediatric neurosurgical emergency treatment. BLI: The bioluminescent GP signal was measured using an IVIS Spectrum/CT instrument (Perkin Elmer). BLI was quantified by drawing of regions of interest (ROIs), with data expressed as photon flux (p/sec). MRI: An undersampled radial FLASH pulse sequence was used with a 3T Prisma Fit MR scanner (Siemens). Statistical Analysis: Result were calculated and subjected to statistical analysis using multivariate regression (PROC MIXED, SAS 9.4).

Results: BLI revealed that lower injection speeds and lower volumes minimize cell outflow outside the ventricular system, while having little effect on the distribution within the ventricular system. Real-time MRI demonstrated that SPIO-labeling significantly alters rheological properties of glial progenitor suspension such that, even at high speeds and high volumes, outflow beyond the ventricular system was reduced. Further detail analysis revealed that the cell origin (human vs. mouse), type of catheter (one-hole vs two-sided hole) and length of the catheter also had substantial effect on the distribution of GPs.

Conclusions: Infusion speed, volume and SPIO labelling strongly influence the biodistribution of glial progenitors injected in a 3D model of piglet cerebral ventricles. Use of 3D model was essential for cost-effective optimization of the intraventricular delivery before it is clinically translated.
Thalamic neurodegeneration after focal cortical stroke in rats does not induce thermal or mechanical hypersensitivity
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The aim of this study was to find out, whether the death of thalamic neurons in the cortical stroke results in sensitization to pain. Central post-stroke pain has been occasionally reported on stroke patients and it has been associated with the death of thalamic neurons. On the other hand, activated microglial cells have been shown to induce chronic pain after *in vivo* spinal cord injury. However, the association of neuronal death and accumulation of activated microglia in thalamus with chronic pain after cortical stroke has not been confirmed *in vivo*.

We used adult male Sprague Dawley rats (250-300 g) which had ad libitum access to food under a 12-h light-dark cycle. 30 animals were distributed to three balanced groups (n=10), intact, sham-operated and stroke, based on their baseline response to thermal pain. We used a 90 minute distal middle cerebral artery occlusion (MCAo) stroke model, because it results in small strokes more relevant to human pathology and infarct core area is restricted to ipsilateral cortex. Rats were anesthetized with i.p. chloral hydrate 0.4 g/kg. Post-operative pain was treated with s.c. carprofen 5 mg/kg before recovery.

We used two methods to evaluate response to pain caused by thermal and mechanical stimuli: Hargreaves' Test and Von Frey test, respectively. These tests measure reaction time to withdrawal of the stimulated paw. The tests were performed on days 3, 14 and 28 after surgery. The animals were perfused transcardially with 4 % paraformaldehyde solution on day 32. 5 μm coronal paraffin sections were cut and stained with mouse anti-NeuN (1:200, Millipore) or mouse anti-CD68 (1:500, AbD Serotec) antibodies to obtain neuronal death and accumulation of microglia/macrophages (M/M cells) in the thalamus, respectively. The cells were counted (Image Pro Plus 7.0), and results were analyzed with one-way ANOVA. The results from Hargreaves' test were analyzed with repeated measures ANOVA, and the results from von Frey test with Kruskal-Wallis test, because some measurements exceeded the cut-off time.

There was a significant difference in neuronal loss between the groups (F_2;20=11.627, p<0.001, one-way ANOVA). In the stroke group, number of NeuN+ neurons decreased significantly compared to Sham-operated and intact rats (Games-Howell: p<0.01 and p<0.05, respectively). Average number of neurons in the ipsilateral thalamus in the stroke group was 73 % of the number in the contralateral thalamus, whereas in both control groups it was close to 100 %. In addition, we observed a significant accumulation of activated M/M cells in the thalamus, of stroke group compared to control groups (F_2;22=15.029, p<0.001, one-way ANOVA. p<0.05, Games-Howell). However, there was no significant difference in the reaction time to thermal or mechanical pain between the groups. Therefore, we conclude that neuronal death and microglial accumulation in thalamus alone does not induce hypersensitization to pain.
HH exposure. Further, we investigated its possible role during HH-induced cognitive impairment in a rat model system. Our study suggested several interesting facts, the expression of active TGF-β and downstream signaling is present as early as 1-day post-HH concomitant with, neuronal death, microglial and astrocyte activation in vivo. The inhibition of this pathway attenuated spatial memory deficits as revealed by Morris water maze test. Chronic inhibition during normoxic conditions was, however, counterproductive, suggesting paradoxical nature of signaling through the TGF-β receptor. Employing primary astrocyte culture, we observed that TGF-β expression occurs in response to hypoxic conditions per se but the generation of active form and downstream signaling was, however, dependent on additional cues – manifesting in intact brain tissue during hypoxic conditions. Strikingly, pro-coagulant proteins are detected in the plasma employing 2D proteomics analysis, previously not only known to activate TGF-β but also associated with various brain pathology. These results, taken together, suggest a likely therapeutic effect of TGF-β inhibition on HH induced memory deficit besides suggesting this molecule as a key node integrating intrinsic hypoxia and injury responses.

P II - 6-13
Effects of CDNF on the alpha-synuclein fibrils model in young and aged rodents
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Parkinson’s disease is a progressive neurodegenerative disorder that affects over 7 million people worldwide. α-synuclein (α-syn) is mutated in certain patients with familial forms of Parkinson’s disease, and it is a major component of insoluble protein aggregates called Lewy bodies, present in almost all patients. Injection of preformed fibrils consisting of purified α-syn (PFFs) into rodents is a promising model of the disease due to the presence of Lewy bodies.

Cerebral dopamine neurotrophic factor (CDNF) has been shown to be neurorestorative in neurotoxin models of Parkinson’s disease. Importantly, it is able to rescue neurons from endoplasmic reticulum-induced cell death, a process associated with the aggregation of α-syn. The objective was first to create an α-syn-based model of Parkinson’s disease in our laboratory using PFFs (from mouse) and then to test whether CDNF can have a positive effect on this model.

The main methods used are stereotaxic injections, behavioural tests of motor deficits, immunohistochemistry/immunofluorescence, and HPLC; this was performed both in young and aged rodents with time points ranging from 2 hours to 6 months.

In general, we observed some motor deficits in this model, and we continue to explore how CDNF may affect the outcome of α-syn spreading and aggregation, as well as the dopamine system.

P II - 6-14
Implementation of neural networks to quantify substantia nigra dopamine neurons and Lewy bodies
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Question: Unbiased estimates of neuron numbers within substantia nigra are important for experimental Parkinson’s disease models. Although the widely used unbiased stereological counting techniques with optical fractionation are accurate, these techniques are extremely laborious and time-consuming. The development of artificial neural networks and deep learning has enabled the implementation of machine learning for automated
cell counting. The advantages of computerized counting are reproducibility, elimination of human errors, and fast high-capacity analysis. We implemented whole-slide digital imaging and convolutional neural networks (CNN) to count dopamine neurons and Lewy bodies in mouse and rat brain.

Methods: After immunohistochemistry, digital whole-slide images of brain sections were acquired at 0.22 µm/px resolution. We trained a CNN algorithm on Aiforia® platform using 528 megapixels of image data to recognize TH-positive neuron cell bodies in the digital images and validated against published data and independent human observers.

Results: The algorithm performance was validated against stereology (Pearson correlation of 0.9, P<0.0001; R² was 0.819; n=44) and against manual neuron cell body counts by two independent observers in regions that were not included in the training data (Pearson correlation 0.98, p<0.001; R²=0.95; n=26). The sensitivity, specificity and F1-score for the algorithm were 88.5% (CI95: 85.5–91.4%), 87.8% (CI95: 84.9–90.7%), 88.2% (CI95: 85.3–91.0%), respectively. We also trained an algorithm to detect phosphorylated alpha-synuclein (pSer129), a marker for Lewy bodies.

Conclusions: The algorithms developed on Aiforia® are robust tools for cell counting in rat and mouse brain sections enabling fast and high-capacity analytics for experimental studies of Parkinson’s disease. Under the Aiforia® platform, the algorithms can be trained to detect and quantify different markers to help advance research on other neurodegenerative diseases.

P II - 6-15
Neuroprotective efficacy of microRNA biogenesis pathway stimulation in primary dopaminergic neurons
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Parkinson’s disease (PD) is a common neurodegenerative disorder characterized by progressive loss of dopaminergic neurons. Recently we have demonstrated that disturbances in biogenesis of microRNAs, small regulatory non-coding RNAs, may be implicated in PD pathology. We have shown that selective homo- and heterozygous deletions of microRNAs processing enzyme Dicer in vivo cause development of PD-like symptoms. Additionally, pharmacological stimulation of microRNA biogenesis with enoxacin results in improved survival of primary dopaminergic neurons and attenuates their vulnerability to endoplasmic reticulum stress. We have also established an original experimental system utilizing specifically defined conditions which, combined with high content imaging and in-house developed automated image analysis workflow, allows us to model alpha-synuclein aggregation and neurodegeneration induced with pre-formed fibrils and lentivirus vector-mediated alpha-synuclein expression in cultured dopaminergic neurons.

Here we explore the role of microRNA biogenesis pathway in neuronal maintenance and protection from different stressor-induced insults by manipulating microRNA maturation pathway with several approaches. First, to stimulate microRNAs maturation in primary neuronal cultures, we expressed Dicer and other proteins involved in microRNA biogenesis using lentiviral vectors, and assessed their effects on survival and protection of midbrain dopaminergic neurons from several PD-associated stressors, such as pre-formed alpha-synuclein fibrils, thapsigargin, and 6-OHDA.

Second, we investigated the role of microRNAs in dopaminergic neurons via impairing their maturation process by neuron-specific homo- and heterozygous deletion of Dicer using LoxP/Cre-recombinase system in neuronal cultures. Obtained dopaminergic neurons are further tested for the ability to withstand stress conditions. We are also utilizing neuron-specific CRISPR/Cas9 gene editing to target Dicer and its interaction partners to study their effect on dopaminergic neuronal survival and resistance to stress.
In summary, our data assess the role of Dicer-dependent microRNAs biogenesis in supporting survival and stress resistance of primary neurons and evaluate the possibility of targeting Dicer and its interaction proteins as therapy for PD and, possibly, other neurodegenerative diseases.

**P II - 6-17**

**Degeneration of muscles supplied by the external carotid artery in the intraluminal filament mouse model of middle cerebral artery occlusion**

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**Objectives:** Transient or permanent middle cerebral artery occlusion (MCAO) in mice is the most common form of focal cerebral ischemia, to study pathophysiological mechanisms and interventions. In the surgical procedure the external carotid artery (ECA) is ligated, and restricts the blood flow to the ECA territory. Yet the consequences for the underlying musculature including chewing and swallowing have not been described. Therefore, we studied the effects of restricted blood flow in the ECA territory with multi-spectral optoacoustic tomography (MSOT) on the oxygenation and long-term pathophysiological changes with magnetic resonance imaging (MRI) and histology on the temporal muscle.

**Methods:** C57BL/6 mice underwent 1h of transient MCAO (tMCAO) or sham surgery with ligation of the ECA. MSOT was employed at 30min after surgery to assess acute changes in tissue oxygenation in the temporal muscles. Time-of-flight angiograms were acquired to examine blood flow in large arteries and in T2 maps region-of-interest were drawn over the temporal muscles to observe microstructural changes after 24h and 48h. Histology of the whole mouse head was used to assess pathological changes in the entire ECA territory.

**Results:** Ligation of the ECA resulted in an acute decrease in tissue oxygenation in the left temporal muscle in around 70% of sham and tMCAO animals, while time-of-flight angiogram showed an arrest of the blood flow in the ECA. Susceptible mice of both groups exhibited increased T2-relaxation times at 24 and 48h in the affected muscle with similar values. Histopathology revealed myofibre degeneration and interstitial edema in the temporal muscle and other tissues underlying the ligated ECA. Also the histopathological changes correspond to increased T2-relaxation times, whereas the contralateral side was unaltered.

**Conclusions:** ECA ligation leads to degenerative changes in the muscles of the ECA territory, while a potential impact on outcome needs to be considered in this stroke model.

**P II - 6-18**

**Modified extracellular matrix can support post stroke neuroplasticity**

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**Objectives:** Extracellular matrix (ECM) of the brain provides a highly dynamic scaffold to regulate multiple functions of neurons. By incorporating a variety of secreted molecular guidance factors, ECM provides essential control over synaptogenesis and plasticity driven neural rewiring. On the other hand, ECM scaffolds are pivotal for the synapse stabilization and thus are crucial for the maintenance of functional connectivity. We aimed to understand whether the modification of macromolecular ECM aggregates, called perineuronal nets (PNNs), can help neural rewiring after exposure to stress conditions.
Methods: Post stroke ECM alterations were studied in context of ischemia-reperfusion induced in mice using the temporal middle cerebral artery occlusion (tMCAo) method. To provide a reliable quantification of ECM ultrastructure, we have developed a novel approach combining superresolution structured illumination imaging (SR-SIM) and mathematical reconstruction. Network activity and plasticity were investigated in vitro using multiple electrode arrays (MEAs) and custom developed morphological assays.

Results: Our results indicated that focal cerebral ischemia induces partial depletion of PNNs and that mild hypoperfusion not associated with ischemic injury can induce ultra-structural rearrangements in visually intact meshworks. Importantly, the observed rearrangement of ECM in the perinfect cortex was not associated with neuronal cell death, but correlated with reactive gliosis. Furthermore, ECM digestion in vitro induced profound changes in network connectivity and activity patterns.

Conclusion: In line with the activation of neural plasticity under stress stimuli, we suggest that subtle modification of ECM can support post injury neurologic recovery. Thus, brain ECM comprises a promising target for future restorative therapies.

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P II - 6-19
Alterations in Brain tissue redox status and blood-brain barrier permeability in presymptomatic hSOD1 G93A ALS rats
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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder affecting both the motor and cognitive domains of the central nervous system (CNS). Mutations in the Cu,Zn-superoxide dismutase (SOD1) cause 20% of familial ALS and provoke formation of intracellular SOD1 aggregates and copper and zinc unbinding, leading to pathological intracellular changes and neurodegeneration. Post mortem and animal model studies indicate blood-brain barrier (BBB) disruption, elevated production of reactive oxygen species, and perturbed transition metal homeostasis as major contributors to disease pathology. In order to further clarify the pathophysiological mechanisms and to identify early changes in the disease we investigated the pathological changes in motor (brainstem) and non- motor (hippocampus) brain centers of a transgenic hSOD1 G93A rat model of ALS, at presymptomatic and symptomatic stages of the disease.

The brain tissue redox status and BBB permeability in hSOD1 G93A rats were investigated by in vivo EPR spectroscopy using the aminoxyl radical 3-carbamoyl proxyl (3CP). Pharmacokinetic modeling of 3CP reduction in vivo in the head of presymptomatic and symptomatic hSOD1 G93A rats indicated an altered brain tissue redox status and possible BBB disruption in these animals. Altered brain redox status in hSOD1 G93A rats was confirmed by biochemical assays showing increased nitration, superoxide radical production, lipid peroxidation and SOD2 activity, and a decreased SOD1 activity in brainstem and hippocampus tissue homogenates of these animals. BBB disruption was confirmed by MRI experiments showing presence of BBB breakdown already in presymptomatic animals, with further spreading of BBB damage in symptomatic rats. Topographic and quantitative analysis of the tissue elemental composition by X-ray fluorescence imaging revealed decreased P and increased Ca, Cl, K, Ni, Cu and Zn in the brainstem, and higher levels of Cl, Ni and Cu, but lower levels of Zn in the hippocampus of symptomatic hSOD1 G93A rats.
These results bring new insights into the alterations in tissue elemental homeostasis during disease development and progression in motor as well as in non-motor CNS structures, and indicate a disturbed brain tissue redox status and BBB disruption as early markers of the disease.

P II - 6-20
Effect of perineuronal net inhibitor on memory retention in mice
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Perineuronal nets (PNNs) are lattice-like extracellular matrix structures composed of hyaluronan (HA), chondroitin sulfate proteoglycans (CPSGs), tenascin and link proteins, which surround the surface of the soma and dendrites, and in some cases the axonal initial segments. The PNNs are responsible for synaptic stabilization in the adult brain, and disruption of PNNs may reactivate neural plasticity.

In this study, we investigated the possibility of memory prolongation by reduction of PNN formation using a PNN inhibitor (PNNi). To examine the effect of the PNNi on memory, C57BL/6 adult mice (n=16) were fed by chow containing 5% (w/w) PNNi for 6 months, in a dose 250 mg/mouse/day. The memory retention was tested using novel object recognition test (NOR) and spontaneous alternation test (SA).

NOR test was performed after 2, 3, 6 and 7 months with intervals between the NOR sessions of 3h and 24h. An improvement in NOR score was found in animals treated with PNNi during the all treatment period compared with control group. However, 1 month after the end of the treatment, the effect of PNNi did not persist. This suggests a constant low level of PNNs is required for a persistent memory enhancement.

SA test was performed after 2 and 6 months. Spatial memory and activity have been determined, no significant differences between PNNi treated group (n=16) and control groups (n=8) were observed.

To determine if PNNi affects motor coordination, we used two types of tests including grip and rotarod tests. After 6 months of PNNi treatment, we did not observe any differences in motor skills between the treated (n=8) and control animals (n=4).

These findings suggest that manipulation of PNNs by oral administration of PNNi may increase plasticity and might offer a novel therapeutic approach to the treatment of memory loss in neurodegenerative disorders such as Alzheimer’s disease or dementia.

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P II - 6-21
Proliferation and differentiation of NG2-glia following different types of brain disorders
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NG2-glia, a fourth major glial cell population, are present in the adult central nervous system and display distinct morphology, antigens and functions from other mature glial cell types. Recently, many studies have shown that these cells are multipotent in vitro and they also display wide differentiation potential under pathological conditions in vivo, where they give rise predominantly to reactive astrocytes.

The aim of this study was to identify the rate of proliferation and differentiation after different types of brain disorders, such as global and focal cerebral ischemia (GCI, FCI), cortical and hippocampal stab wound (SW COR/HIP) and demyelination (DEMY). We used transgenic Cspg4-cre/CAG-tdTomato mice, which after administration of tamoxifen express red fluorescent protein (tdTomato) in NG2-glia and cells derived therefrom. Proliferation and differentiation potential of tdTomato positive (tdTomato+) cells in sham-operated mice (controls) and those after injury were determined by immunohistochemistry - e.g. proliferating cell nuclear antigen (PCNA), glial fibrillary acidic protein (GFAP). FCI was induced by middle cerebral artery occlusion, GCI by carotid occlusion with hypotension, SW by sagittal cortical cut and DEMY by feeding mice with copper chelator cuprizone. To determine the phenotype of tdTomato+ cells, the coronal brain slices were used from controls and mice 3 and 7 days after FCI, GCI, SW COR/HIP or DEMY. The percentage of cells that were double positive for tdTomato and PCNA or GFAP was estimated.

We have shown that NG2-glia increase their proliferation rate after all types of brain disorders, such as ischemia, cortical injury as well as demyelination. In case of acute injuries (FCI, GCI, SW) the highest proliferation rate was observed three days after the insults and even seven days after injury the proliferation rate was increased compared to controls. In case of DEMY the proliferation increased seven days after withdrawal of cuprizone diet. In accordance with previous studies, differentiation of NG2-glia into astrocytes was almost absent in uninjured nervous tissue, however, the type of injury influenced NG2-glia differentiation towards astrocytes.

Taken together, increased proliferation of NG2-glia is the typical feature after all types of pathological conditions, while differentiation into astrocytes depends on the type of injury. Supported by the Grant Agency of the Czech Republic: GACR 17-04034S, GAUK 618216.

P II - 6-22
hiPSC cell models for the validation of small molecule inhibitors of the TRPM4 ion channel – a novel target for multiple sclerosis
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Multiple sclerosis (MS) is the most frequent chronic inflammatory disease of the central nervous system (CNS), leading to axonal demyelination and progressive neuronal degeneration. While progress has been made in treating the inflammatory processes of MS, neuronal loss in the CNS is not well understood and there is no curative treatment available yet.
Recently, the ion channel transient receptor potential melastin 4 (TRPM4) has been shown to be involved in the process of inflammation-mediated neurodegeneration. Activation and misexpression of TRPM4 contributes to neuroaxonal damage in the CNS, without modulation of the immune response in the animal model of MS (experimental autoimmune encephalomyelitis; EAE). Furthermore, genetic knock out or unspecific blockage of TRPM4 led to an amelioration of the EAE disease course and an increased resistance of mouse neurons to glutamate-induced excitotoxicity.

Previously, the suitability of TRPM4 as a therapeutic target was established and we have identified potent and selective small molecule inhibitors of the TRPM4 channel in a high-throughput screening approach. Hits cluster in five hit series and initial structure-activity-relationships (SAR) have been established. Validated hits have further been optimized in hit-to-lead programmes and are currently being extensively validated in various in vitro and in vivo assays using human cell lines and primary mouse neurons. We aim to demonstrate the neuroprotective effect of lead candidates in human glutamatergic neurons generated from induced pluripotent stem (iPS) cells. For this purpose, we have established protocols for their differentiation into cortical neurons and were able to show an upregulation of TRPM4 and NMDA receptors during the differentiation process. Ongoing studies aim at further characterizing functional integrity of the cellular products and validation of the efficacy of the hits in TRPM4 wild-type and CRISPR-cas9 knock-out cells.

P II - 6-23
Dementia and beyond – A new collaborative research initiative between Kobe and Germany – The C-CNS Project
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Objectives: The origins of the C-CNS Project are due to the similarity between Germany and Japan, in that both countries are undergoing demographic changes and aging populations. As a consequence there is an increasing incidence of age-related cerebrovascular diseases for which therapeutics need to be developed.

Material & Methods: The partners of the C-CNS project are (1) Fraunhofer Institute for Molecular Biology & Applied Ecology – Screening Port (Fraunhofer-IME-SP) in Hamburg, Germany which offers access to industry standard pre-clinical drug discovery know-how and complementary infrastructure. The overarching goal of the research activities at Fraunhofer-IME-SP are the development of diagnostic tools and therapies for various human diseases and (2) Institute for Biomedical Research and Innovation (IBRI) in Japan which carries out fundamental research mainly in three domains, namely clinical immunology, aging, and regenerative medicine, with research oriented towards the development of diagnostic and therapeutic methods.

Results: Following the call by the German Ministry of Education and Research (BMBF) to fund a collaborative Asia-Pacific Regional project in 2016, IBRI linked up with Fraunhofer IME to combine their joint research interest in cerebrovascular disorders and cell treatments. Their common plan focused on inflammation and dementia including a data-generating pilot research and to translate any findings into early stage clinical trials.

Conclusion: The C-CNS project is a fit-for-purpose response to the high socio-economic pressure caused by the increase in age-related diseases and aims to find sufficient treatment options. An inauguration meeting has been held and a number of activities are being planned which will include scientific exchange missions and training workshops in Germany and Japan.