Poster Session 5: Stroke and brain hemorrhage

P I - 5-1

Protein Z plasma levels in ischemic stroke patients undergoing thrombolysis: a pilot study

*A. Slomka[1], U. Rosinska[2], M. Sury[3], M. Wiszniewska[2], E. Zekanowska[1]

[1]Nicolaus Copernicus University in Torun, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Department of Pathophysiology, Bydgoszcz, Poland
[2]Stanislaw Staszic Specialist Hospital, Neurology and Stroke Care Unit, Pila, Poland
[3]Stanislaw Staszic Specialist Hospital, Department of Laboratory Medicine, Pila, Poland

Question: Protein Z (PZ) inhibits the activity of coagulation factor Xa, thus presents anticoagulant properties. A few studies have researched the levels of PZ in patients with ischemic stroke (IS) but the results are conflicting. This pilot study aimed to evaluate the plasma levels of PZ and autoantibodies to PZ (anti-PZ), and the dynamics of changes in their levels after IS.

Methods: Twenty two IS patients (13 men and 9 women with the mean age 68±11 years) who underwent intravenous thrombolysis with alteplase were included in the study. Venous blood sample of 5 mL was collected from each individuals. PZ, anti-PZ IgM, and anti-PZ IgG plasma levels were measured before and 7, 30, and 90 days after treatment using high-sensitivity ELISA kits from Hyphen BioMed (France). The Friedman test was used to verify whether the differences in the levels of PZ, anti-PZ IgM, and anti-PZ IgG at four time points were statistically significant.

Results: All patients were diagnosed with first-ever IS and received alteplase at a dose of 0.9 mg/kg body weight. The median values of PZ at baseline and at day 7, 30, and 90 were 2.53 µg/mL, 2.59 µg/mL, 2.44 µg/mL, and 2.43 µg/mL, respectively. No statistically significant differences were observed between these values (p=0.19). Moreover, none of the IS patients were shown to have positive results for anti-PZ IgM and anti-PZ IgG before and 7, 30, and 90 days after treatment.

Conclusions: Three conclusions may be drawn from our pilot study. First, the levels of PZ seem to be relatively stable in patients treated with thrombolysis for IS. Second, these results show that IgM and IgG isotypes of anti-PZ autoantibodies are not present in IS individuals. Third, there is now a critical need to confirm our observations in a larger group of IS patients.

P I - 5-2

Inhibition of G9a sensitizes neuronal cell death via increasing DNA damage under ischemic condition

*S. Baik[1]

[1]National university of Singapore, Physiology, Singapore, Singapore

G9a is part of the histone methyltransferase family in eukaryotic species. It's canonical function is to regulate di- and tri-methylation of H3K9, which causes the suppression of target genes. But it also induces methylation of non-histone protein,p53, as well as H3K9. Moreover G9a is a positive regulator of homologues recombination (HR) of DNA and is required for DNA damage repair. Ischemic stroke is well known to cause severe brain damage and mortality. DNA damage is also one of characteristic features of cerebral ischemia induced neuronal cell death. Here, we found that inhibition of G9a worsens neuronal cell death under oxygen glucose deprivation (OGD) condition. G9a is up-regulated under ischemic conditions. Transcriptional and pharmacological inhibition of the G9a increases OGD induced DNA double strand break and apoptosis.

P I - 5-3

Response to Leukemia Inhibitory Factor is dependent on age and sex in a rat model of large vessel occlusion

*K. Pennypacker[1], L. Collier[1], S. Goodwin[1], S. Davis[1]

[1]University of Kentucky, Neurology, Lexington, United States

Leukemia inhibitory factor (LIF) is an anti-inflammatory cytokine that confers neuroprotection when administered systemically after permanent middle cerebral artery (MCAO) occlusion in three month old male Sprague-Dawley rats. LIF signaling activates the transcription factor MZF-1 via the PI3K/Akt pathway. MZF-1 transcriptional activity increases expression of the following antioxidant genes: peroxiredoxin IV and metallothionein III (oligodendrocytes), and superoxide dismutase 3 (neurons). Intravenous administration of LIF at 6, 24, and 48 h post-stroke results in significantly reduced infarct volume and increased motor recovery in young male rats at 72 h post-MCAO. This treatment produced partial motor recovery in aged (18 mo) female rats and no recovery in aged males. In young males and aged females, LIF treatment ameliorated the post-stroke inflammatory response that originates in the spleen. This treatment in young male rats results in a smaller reduction in spleen weight and lower splenic levels of the inflammatory cytokines interferon gamma (IFNγ) and CXCL10 at 72 h post-stroke. In aged female rats, the splenic weight is significantly increased and splenic IFNγ/CXCL10 levels are significantly reduced. This treatment regimen increased expression of LIFR in the ipsilateral hemisphere of the brain at 72 h after stroke in young males but not aged rats of either sex. In uninjured brain tissue, LIFR is localized to neuronal nuclei. During brain injury, LIFR migrates to the cell membrane. This translocation occurs in the ischemic tissue of both aged and young rats. The LIFR acts as the transcellular transporter across endothelial cells. Immunohistochemistry shows more LIFR and endothelial protein, von Wildebrand Factor, in young male brains relative to aged brains after stroke. These observations suggest that the transport of LIF is reduced in aged brain thus resulting in little or no neuroprotection. These results demonstrate that the neuroprotective and anti-inflammatory effects of LIF are dependent on both age and sex.

P I - 5-4

Imaging of brain reorganization after experimental stroke by repetitive two-photon microscopy

*S. Valero Freitag[1], A. Lourbopoulos[1], R. Sienel[1], F. B. Seker[1], U. Mamrak[1], J. Cramer[2], A. Liesz[2], F. Hellal[1], N. Plesnila[1]

[1]Institute for Stroke and Dementia Research, University of Munich Medical Center, Experimental stroke, Munich, Germany
[2]Institute for Stroke and Dementia Research, University of Munich Medical Center, Stroke Immunology, Munich, Germany

Introduction: Correlation between structural reorganization and functional long-term outcome after ischemic damage is up to now not completely understood. This is in particular due to the lack of appropriate repetitive imaging tools and animal models depicting long-term behavioral deficits. Here we aimed to develop a protocol optimizing the most critical points to study longterm stroke recovery using the transient Middle Cerebral Artery occlusion mouse model (tMCAo).

Methods: Male C57BL/6N mice (6-8 weeks of age) received multiple stereotaxic injections of AAV2-EGFP to sparsely label neurons in the region of the sensorimotor cortex followed by cranial window implantation. One month after injection, mice were randomly subjected to 1h tMCAo or sham surgery. Over the next 14 days mice received nutritional support by daily injections of 1 ml NaCl and 20% glucose. Two-photon imaging was performed under light sedation using 0.05mg/kg medetomidin in combination with 1% isofluorane. General and focal deficits were assessed using modified Neurological Severity Score (mNSS). Additionaly Gad65-GFP animals and FITC-dextran were used for interneurons and vessel labeling, respectively.

Results: Our optimized post-operative care protocol resulted in low mortality (<10%) of mice subjected to tMCAo which yet showed sustained neurological deficits up to two months after stroke. We corrected commun artifacts observed during imaging by embedding the cranial window glass in the skull bone directly in contact with the

brain parenchyma to prevent brain pulsations due to heartbeat. Moreover, we administrated Dexamethasone (0.5 mg/kg) during surgery to avoid edema formation. This allowed imaging of fine neuronal structures including dendritic spines and performing time lapse imaging of circulating blood cells. The light sedation regimen as an alternative to deep anesthesia permitted repetitive imaging without depressing brain activity.

Conclusion: Here we provide a live-imaging protocol to study longitudinal changes in various cortical structures such as blood vessels, neurons and dendritic spines. Combined with an optimized stroke model for longterm survival, it enables to relate these changes to the long-term functional outcome after ischemic stroke to uncover new potential therapeutic targets.

P I - 5-5

Age-related differences in recovery of cognitive function following stroke to the prefrontal cortex: BDNF-mediated reopening of a critical window for stroke recovery

*J. Houlton[1], L. Zhou[1], E. Gowing[1], D. Barwick[1], A. Clarkson[1]

[1]University of Otago, Dunedin, New Zealand, Anatomy, Dunedin, New Zealand

Question: Stroke remains a leading cause of adult death and disability worldwide. Recently, we have established an animal model of stroke that results in delayed impairment in spatial memory, allowing for us to better investigate cognitive deficits. Young and aged brains show different recovery profiles after stroke, therefore, we assess aged-related differences in post-stroke cognition. As neurotrophic support diminishes with age, we also investigated the involvement of brain-derived neurotrophic factor (BDNF) in these differences, a neurotrophin critical for post-stroke recovery of motor function.

Method(s): Young (3-6 month old) and aged (16-24 month old) mice were trained in operant touchscreen chambers to complete a visual pairwise discrimination (PD) task. Stroke (young, *n=11;* aged, *n*=25) or sham (young, *n=12;* aged, *n*=7) surgery was induced using the photothrombosis model to induce a bilateral prefrontal cortex stroke. Five days post-stroke, aged control animals were treated with intraperitoneal saline (*n*=8) or intracerebral hydrogels loaded with IgG-Fc (*n*=11). Remaining aged stroke animals were treated with hydrogels loaded with TrkB-Fc (a BDNF decoy, *n*=8). Following treatment, animals underwent a reversal task to identify stroke-induced cognitive deficits 3-4 weeks post-stroke.

Result(s): Assessment of sham animals using the Kaplan-Meier survival curves and log-rank analyses demonstrated that aged mice are more impaired on PD reversal learning compared to young controls ($\chi2=10.76$, $df=1$, $p=0.001$). Stroke to young mice revealed no impairment on the reversal task ($\chi2=0.332$, $df=1$, $p=0.564$). In contrast, stroke to aged mice facilitated a significant improvement in the reversal learning ($\chi2=16.02$, $df=1$, $p=0.0011$), which was dampened in the presence of the BDNF decoy, TrkB-Fc ($\chi2=3.137$, $df=1$, $p=0.2084$). Further assessment revealed aged stroke control animals required significantly less trials ($p=0.0034$) and correction trials ($p=0.0131$) to master the reversal task, relative to aged shams. TrkB-Fc treatment, again, dampened this effect, leaving animals to perform similarly to aged sham animals (total trials, $p=0.2483$; total correction trials, $p=0.6866$).

Conclusion: Our findings support age-related differences in recovery of cognitive function after stroke. Interestingly, aged stroke animals outperformed their sham counterparts, suggesting reopening of a critical window for recovery. Since TrkB-Fc treatment appeared to diminish this effect, we propose this mechanism may be mediated by BDNF. To further investigate the therapeutic potential of these findings, additional experiments treatments are being assessed using either systemic administration of the BDNF-inducing AMPAkine, CX1837, or hydrogels delivery of exogenous BDNF.

P I - 5-6

Identifying Changes in Axonal Connectivity following Prefrontal Cortex Stroke in Mice: Impaired Thalamic Input.

*D. Barwick[1], M. T. Joy[2], E. Gowing[1], S. T. Carmichael[2], A. Clarkson[1]

[1]University of Otago, Anatomy, Dunedin, New Zealand
[2]University of California, Los Angeles, Neurology, Los Angeles, United States

Question: Stroke is a leading cause of death and disability worldwide. One third of stroke survivors will suffer cognitive impairments, as neuronal networks essential for normal processing become disrupted. The prefrontal cortex (PFC) is an important hub for higher cognitive functioning. We have recently established a model of PFC stroke resulting in delayed impairments in spatial memory and attention. Two regions thought to be involved in higher functioning are the prelimbic region (PL) and hippocampus (CA1). To further investigate the mechanisms underlying delayed cognitive impairment, we performed tract-tracing experiments to assess temporal changes in neuronal connectional maps after PFC stroke.

Method(s): Focal ischemia (*n=27*) or sham (*n=9*) was induced in mice (5-7 month old) by photothrombosis to the PFC bilaterally. To investigate changes in connectivity following stroke two different fluorescent, retrograde tracers were injected into either the PL or CA1. Acute and chronic changes in connectivity were assessed at 1-week (*n=9*) and 4-weeks post-stroke (*n=9*). A second chronic group was added (*n=9*) receiving a novel intervention from 3-days post-stroke. Perfusion fixed brains were collected 5-days following injections, then coronally sectioned (50um) and imaged using a confocal microscope. The number of projections into the prelimbic or hippocampus within predefined regions of interest were quantified using Imaris and differences assessed between treatments groups using one-way ANOVA.

Result(s): Preliminary quantification cell numbers, indicates a time-dependent and progressive loss of cells projecting to the PL from both the medial dorsal (*MD, p=0.0933*) and nucleus reunions (*RE, p=0.0003*) thalamic nuclei from 1 to 4-weeks post-stroke. Treatment with the novel intervention appears to protect against the progressive loss of connections observed in both regions (*MD, p=0.0621; RE, p<0.0001*). Other regions of interest investigated do not show this same loss of connectivity across the different treatment groups.

Conclusion: The progressive loss of connectivity between the thalamus and PL observed in the current experiment matches the time point where we previously observe delayed impairments in spatial memory following PFC stroke. In order to confirm that these observations are causally linked we are performing intervention studies to assess whether preventing the loss on connections from occurring also prevents the delayed impairments in spatial memory.

P I - 5-7

Description of a novel phosphodiesterase-3 inhibitor protecting mice from ischemic stroke independent from platelet function

*M. Bieber[1], J. Volz[2], M. K. Schuhmann[1], V. J. Rao[3,4], B. Nieswandt[2], M. Pham[5], G. Stoll[1], C. Kleinschnitz[1,6], P. Kraft[1,7]

[1]University Hospital Würzburg, Neurology, Würzburg, Germany
[2]University of Würzburg, DFG Research Center for Experimental Biomedicine, Würzburg, Germany
[3]Indian Institute of Chemical Technology, Organic Chemistry Division II, Hyderabad, India
[4]National Institute of Pharmaceutical Education and Research, Hyderabad, India
[5]University Hospital Würzburg, Neuroradiology, Würzburg, Germany
[6]University Hospital Essen, Neurology, Essen, Germany
[7]Klinikum Main-Spessart, Neurology, Lohr, Germany

Question: Acetylsalicylic acid and clopidogrel are the two main anti-thrombotic drugs for secondary prevention in patients with ischemic stroke (IS) without indication for anticoagulation. Due to their limited efficacy and

potential side effects novel anti-platelet agents are urgently needed. Cilostazol, a specific phosphodiesterase (PDE)-3 inhibitor, protected from IS in clinical studies comprising mainly Asian populations. Nevertheless, the detailed mechanistic role of PDE-3 inhibitors in IS pathophysiology is hardly understood. In this project we analyzed the beneficial effects and pathophysiologic mechanisms of novel PDE-3 inhibitors in a mouse model of focal cerebral ischemia.

Methods: Focal cerebral ischemia was induced by transient middle cerebral artery occlusion (tMCAO) in 6 to 8 week old male C57Bl/6 wild-type mice receiving various innovative and only recently described new PDE-3 inhibitors or vehicle 1 h after ischemia induction. Infarct volumes and functional outcomes were assessed between day 1 and day 7, and findings were validated by magnetic resonance imaging. Blood-brain barrier (BBB) damage as well as the extent of thrombosis and local inflammatory response was determined post stroke.

Results: Inhibition of PDE-3 by pharmacological blockade significantly reduced infarct volumes and improved neurological outcome on day 1 and 7 after experimental cerebral ischemia. Reduced BBB damage, less intracerebral thrombus formation and an attenuated local brain tissue inflammation could be identified as potential underlying mechanisms. PDE-3 inhibitor treatment did not increase the number of intracerebral hemorrhages.

Conclusions: The stroke protective effect of novel PDE-3 inhibitors might be an interesting therapeutic target to combat IS via inhibition of thromboinflammatory mechanisms and stabilization of the BBB. Therefore, pharmaceutical inactivation of PDE-3 represents a promising translational approach to combat ischemic brain damage in the future.

P I - 5-8

Spontaneous intracerebral haemorrhage in zebrafish larvae induces translatable pathological and immunological outcomes

*S. Crilly[1], A. Njegic[1], S. Laurie[1], E. Fotiou[1], J. Barrington[1], A. Badrock[1], J. Cave[1], K. Webb[1], H. Young[1], A. Hurlstone[1], A. Parry-Jones[1], S. Allan[1], P. Kasher[1]

[1]The University of Manchester, Manchester, United Kingdom

Questions: The disease mechanisms underlying intracerebral haemorrhage (ICH) are poorly understood despite being the most severe form of stroke with a high global mortality rate. Unfortunately, no specific medications have been identified for ICH patients to date. Modelling ICH pre-clinically has proven difficult and initiation of spontaneous ICH is a characteristic of the human condition that is poorly recapitulated in rodent models. Therefore, there is an urgent requirement to investigate the use of alternative pre-clinical methodologies. Here we describe the use of zebrafish larvae as a useful and potentially powerful *in-vivo* system for translational ICH research. The purpose of this work is to characterise the use of zebrafish larvae as a valid alternative pre-clinical model to study the pathological and inflammatory consequences of bleeding in the brain, in the context of ICH.

Methods: Spontaneous ICH was induced in zebrafish larvae using chemical and genetic approaches. By utilising the transparency of zebrafish larvae in combination with the use of transgenic reporter lines, we assessed various pathological and inflammatory phenotypes post-ICH.

Results: We show that spontaneous ICH results in increased brain cell death and a subsequent locomotor defect. Increased recruitment and activity of *mpeg1*-positive macrophages was observed in the brain following ICH. Increased transcription of the pro-inflammatory cytokine, interleukin-1β (IL-1β) was observed in haemorrhaged larvae. Inhibition of IL-1β, through use of the human IL-1 receptor antagonist Anakinra was incapable of rescuing the cell death and locomotor phenotypes associated with ICH, however a decrease in macrophage recruitment and activation to the site of injury was observed.

Conclusions: Our study shows that key pathological consequences of ICH are evolutionarily conserved to zebrafish; thus strengthening the case that this model organism represents a valuable alternative *in-vivo* system

for the pre-clinical investigation of this devastating disease. Importantly, these data provide compelling evidence to support the hypothesis that IL-1 drives the early neuroinflammatory response following ICH and constitutes a viable therapeutic target.

P I - 5-9

Cell-specific regulation of autophagy in cerebral ischemia–reperfusion injury: Sec22b and Ykt6 exert as autophagosome removers in neurons

*H. Li[1], J. Qian[1], G. Zuo[1], J. Yin[1], X. Duan[1], Z. Chen[1], H. Shen[1], X. Li[1], Z. Yu[1], G. Chen[1]

[1]The First Affiliated Hospital of Soochow University, Department of Neurosurgery & Brain and Nerve Research Laboratory, Suzhou, China

Questions: Ischemia-reperfusion (I/R) injury is accountable for poor outcome of ischemic stroke patients. Compelling evidence demonstrating a dual role of autophagy in stroke. However, the potential mechanism switching the role of autophagy from protection to deterioration is delusive.

Methods: Middle cerebral artery occlusion/reperfusion (MCAO/R) model in adult male rats was established. Cultured neurons and brain microvascular endothelial cell (BMVEC) were exposed to oxygen-glucose deprivation/reoxygenation (OGD/R) to mimic I/R injury *in vitro*. Proteins in neurons under OGD/R stress and non-stress were analyzed by two-dimensional gel electrophoresis and mass spectrometry.

Results: Autophagy induction performed before ischemia reduced infarct volume and ameliorated cognitive and learning ability but have an opposite effect once I/R has occurred. Autophagy induction either before or after I/R surgery dramatically reduced BMVEC dysfunction compared with that in untreated controls. In contrast to BMVECs, although autophagy induction performed before ischemia attenuated neuronal death, post-ischemic autophagy induction aggravated neuronal death. Autophagic flux determination showed that autophagy induction either before or after I/R surgery induced an intact autophagic flux in BMVECs, while autophagy induction after I/R has a damaged autophagic flux in neurons. Mass spectrometry identified Sec22b and Ykt6 as two differentially expressed proteins between OGD/R-treated and normal neurons. There were an increase in the level of Sec22b and a decrease in the level of Ykt6 in OGD/R-treated neurons. Sec22b RNA interference and Ykt6 overexpression reduced infarct size, neuronal death, and inflammation after I/R, while Sec22b overexpression and Ykt6 RNA interference exerted opposite effects, which confirmed Sec22b and Ykt6 as two key factors in neuronal I/R injury. Immunofluorescence assay showed co-localizations of Sec22b/Ykt6 and LC3 in axons in normal neurons, while there was an increase in the co-localization of Sec22b and LC3 and a decrease in the co-localization of Ykt6 and LC3 in OGD/R-treated neurons. Furthermore, over-expression of Sec22b enhanced the distribution of autophagosomes at the axon distal, while overexpression of Ykt6 strengthened the autophagosome cytoplasm distribution. Finally, we found that Sec22b RNA interference and Ykt6 overexpression could not reduce infarct size in the MACO/R model in neuron-specific Atg7 conditional knockout mice, which further proved that Sec22b and Ykt6 participate in brain I/R injury by regulating autophagy.

Conclusion: The special synaptic structure of the neurons highlights the importance of autophagosome transport for an intact autophagic flux in cerebral I/R injury. Sec22b and Ykt6 exert as autophagosome removers. Overmuch Sec22b and loss of Ykt6 in I/R treated-neurons lead to failure in the autophagosome transport, damaging the whole autophagic flux, and finally exacerbating neuronal I/R injury.

P I - 5-10

Targeting platelet GPVI but not α2β1-mediated collagen binding contributes to ischemic stroke in mice.

*M. Schuhmann[1], P. Kraft[1], D. Stegner[2], M. Bieber[1], H. Schulze[2], M. Pham[3], B. Nieswandt[2], G. Stoll[1]

[1]University Hospital Würzburg, Department of Neurology, Würzburg, Germany
[2]Rudolf Virchow Center, DFG Research Center for Experimental Biomedicine, Würzburg, Germany
[3]University Hospital Würzburg, Department of Neuroradiology, Würzburg, Germany

Questions: Platelet collagen interactions at sites of vascular injuries predominantly involve glycoprotein VI (GPVI) and the integrin α2β1. We recently showed that depleting GPVI improves stroke outcome without increasing the risk of cerebral hemorrhage. Until now, it has not been addressed whether platelet α2β1 mediated collagen binding also contributes to the pathophysiology of stroke.

Methods: Focal cerebral ischemia was induced in C57BL/6 and *Itga2-/-* mice by a 60-min transient middle cerebral artery occlusion (tMCAO). Animals were pretreated with anti-GPVI (JAQ1) or antigen-binding fragments (Fab) against the platelet integrin α2β1 (LEN/B). Thrombolysis (iv rt-PA treatment) was applied in anti-GPVI treated animals immediately prior to reperfusion. Stroke outcome, including infarct size and functional deficit was determined on day 1 after tMCAO.

Results: We demonstrate that targeting the integrin α2β1 receptor (pharmacologic; genetic) did neither reduce stroke size nor improve functional outcome on day 1 after tMCAO. In contrast, depletion of platelet GPVI prior to stroke was still safe and effective when thrombolysis with rt-PA was administered.

Conclusions: Our results underscore that GPVI, but not the integrin α2β1, is a promising and safe target in the setting of ischemic stroke.

P I - 5-11

Atlas-based lesion mapping for i*n vivo* MRI and *ex vivo* histology of stroke mice

*F. Wieters[1], N. Pallast[1], G. Fink[1], M. Aswendt[1]

[1]University Hospital Cologne, Neurology, Cologne, Germany

Questions: Stroke lesion location and size determine functional deficits and recovery potential. In stroke mice, lesion size and location are usually determined based on histological stainings or magnetic resonance imaging (MRI). Despite its relevance for stroke research, to date no comprehensive and consistent atlas-based analysis is available, which accurately determines the lesion in different modalities and allows for a cross-modality comparison. Such lesion mapping would, however, not only facilitate the correlation between *in vivo* and *ex vivo* data, but also allow to monitor the individual development of the lesion and hence help to answer the question, which brain areas are most relevant for behavioral deficit and recovery of function.

Methods: We established workflows for *in vivo* T2-weighted MRI and *ex vivo* immunostainings for an ongoing study of currently n=25 C57BL/6J mice (10-12 weeks, male). The mice underwent photothrombosis (PT) surgery (50 mW laser, 1500 ug rose bengal) to induce focal lesions in primary and secondary motor cortex (MOp, MOs) and received 6x T2-weighted MRI (9.4T Bruker) as well as behavior tests (Cylinder, Rotating Beam, Grid Walk) during a 4 week recovery period. For histology, animals were perfused and 20 m cryosections were stained for GFAP. Whole brain slice images were acquired with a Keyence BZ-9000 microscope. All imaging data was registered with the Allen Brain Reference Atlas, which provides a high-resolution atlas and more than 1000 brain regions. For MRI, a processing pipeline was developed in Python 3.6 with algorithms of NiftyReg. Microscopy data were registered using a thin-plate algorithm and manually placed landmarks using 3D Slicer software.

Results: Both workflows are user-friendly, semi-automatic, and time-efficient (20 min per mouse). We validated the accuracy of both workflows by comparison to manually assigned regions of interest by two independent expert users. For both datasets, the registration with the atlas was exact also in cases where the tissue was

strongly distorted due to the stroke lesion. Furthermore, the registration with the atlas outperformed other registration approaches (FSL, Elastix, Ants). We have validated so far MRI at two time points (post stroke days 1 and 27) and GFAP (post stroke day 28). With both methods, we were able to validate that MOp and MOs were affected in all mice. As expected, in the acute phase, the stroke lesion expanded to other regions such as retrosplenial area (RSP) and somatosensory areas (SS). Interestingly, we found a correlation between the number of affected regions and behavioral testing, which we are currently validating in more animals.

Conclusions: The developed workflows using open-source software provide easy-to-use lesion mapping of stroke mouse brains based upon both histology and MR scanning.

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P I - 5-12

Ligature-induced periodontitis does not alter acute outcome or inflammation after experimental stroke in mice.

*C. O'Boyle[1], M. Haley[1], C. Smith[1], S. Allan[1], J. Konkel[1], C. Lawrence[1]

[1]The University of Manchester, Manchester, United Kingdom

Question: Stroke is a major cause of disability and mortality. Poorer outcome after stroke is often associated with concomitant inflammatory and/or infectious diseases. Periodontitis (PD) is an extremely prevalent chronic inflammatory disease of the dental supporting structures and is a prominent risk factor for many systemic disorders, including cardiovascular disease and stroke. While epidemiological studies suggest that PD increases the likelihood of stroke, its impact on stroke severity is poorly understood. Here, we sought to determine the contribution of PD to acute stroke pathology.

Methods: We first characterised a double ligature model of PD in C57BL/6 mice for local and systemic inflammatory responses that could potentially impact stroke outcome. We applied this model for 10 days and subjected mice to a middle cerebral artery (MCA) occlusion via intraluminal filament (fMCAo). We also used an enhanced model of PD by coupling the ligature model with repeated intravenous adminstration of a periodontal-specific bacterial lipopolysaccharide (LPS) in order to better mimic the clinical condition. In this experimental paradigm we occluded the distal MCA via ferric chloride application to induce a thrombotic stroke (dMCAo).

Results: Ligature-induced PD caused robust periodontal bone loss, increased bacterial growth, and increased local inflammatory cell trafficking. Systemically, PD increased circulating levels of the pro-inflammatory cytokines, interleukin (IL)-1 beta, IL-17A, and granulocyte macrophage-colony stimulating factor (GM-CSF), and also primed bone marrow monocytes to produce greater levels of tumour necrosis factor (TNF)-alpha. Despite these changes, however, PD did not alter infarct volume, blood-brain barrier breakdown, or central/peripheral inflammation in mice subjected to fMCAo. Further, addition of a periodontal-specific LPS with ligature-induced PD did not have an effect on ischaemic brain damage, blood-brain barrier breakdown or inflammation after dMCAo.

Conclusions: Overall, these findings suggest that PD alone does not affect acute stroke pathology, at least in the experimental paradigm tested here. It remains to be determined, however, if PD increases the likelihood of stroke, if PD affects long-term recovery after stroke, and also, if in tandem with other co-morbidities PD worsens prognosis post-stroke. Given the high prevalence of PD and the growing incidence of stroke, these are important avenues that require future study.

P I - 5-13

Inhaled nitric oxide as a modulator for vascular inflammation after experimental stroke

*R. Sienel[1], B. Seker[1], N. Terpolilli[1], N. Plesnila[1]

[1]Institute for Stroke and Dementia Research (ISD), Experimental Stroke Research, Munich, Germany

Question: Inhaled nitric oxide (iNO) protects the brain from ischemic injury following cerebral ischemia (Terpolilli et al., Circ Res 2012). So far, selective vasodilatation of penumbral collaterals was suggested to be the main mechanism. The aim of the current study was to investigate whether NO, which is also known to inhibit leukocyte adhesion, affects neuroinflammation after experimental ischemic stroke.

Methods: Middle cerebral artery occlusion (MCAo) was induced in C57BL/6 mice for 60 min. iNO (50 ppm) was initiated at the beginning of reperfusion. Three hours later leukocytes were stained with Rodamine 6G and imaged in the ischemic penumbra by *in vivo* two-photon microscopy. Rolling and adhesion of leukocytes on the vascular endothelium were recorded and analyzed by using IMARIS 3D imaging software.

Results: No rolling leukocytes were observed in sham operated mice. Cerebral ischemia resulted in significant rolling (11 +/- 7 cells/ROI) and sticking (12 +/- 9 cells/ROI) of leukocytes to venular and capillary endothelium three hours after MCAo. In animals receiving iNO the amount of adherent and rolling leukocytes was reduced by 75% ($p<0.05$) and 98% ($p<0.00001$) respectively.

Conclusion: Rolling and sticking of leukocytes to the endothelium of venules and capillaries after cerebral ischemia was almost completely blunted (-75 and -98%, respectively) upon treatment with iNO. In addition to its vasodilatatory activity, this effect of iNO may represent a novel mechanism on how inhaled NO mediates neuroprotection. These findings further support the clinical evaluation of iNO as an acute therapeutic for ischemic stroke.

P I - 5-14

The role of the extracellular matrix laminin-511 in vascular inflammation, blood-brain barrier repair and angiogenesis after stroke.

*H. Thurgur[1], J. Penny[1], E. Pinteaux [1]

[1]University of Manchester, Faculty of Biology, Medicine and Health, Manchester, United Kingdom

Stroke is the leading cause of death and disability worldwide and with limited treatment available, there is an urgent need for new therapeutic strategies that target the repair phase to aid recovery. Interleukin(IL)-1 is a major cytokine implicated in the pathogenesis of stroke, and the blood-brain barrier (BBB) is a critical target of IL-1-induced neuroinflammation. Although IL-1 is thought to contribute to BBB dysfunction, recent studies suggest that IL-1 may contribute to BBB repair beyond the detrimental acute phase of stroke. Furthermore, there is growing evidence that the extracellular matrix (ECM), as a dynamic component of the neurovascular unit (NVU), can respond and influence the microenvironment in the central nervous system (CNS). Specifically, several studies show that laminins exert protective properties on the endothelium and neurones of the CNS. Interestingly, a novel role of the ECM as a regulator of IL-1-induced cerebral inflammation after oxygen glucose deprivation has been demonstrated *in vitro,* and recent evidence has identified laminin (LM)-511 as a key mediator that promotes BBB repair after hypoxia and inflammation *in vitro*. Hence, we hypothesise that LM-511 is a critical regulator of inflammation post-stroke, ultimately contributing to BBB repair through mechanisms of angiogenesis and neurogenesis. We have used a combination of a brain endothelial cell line and primary mouse brain endothelial cells to investigate the role of LM-511 on brain endothelial cell adhesion, proliferation and vascular inflammation. Through our recent studies, we show that LM-511 stimulates endothelial proliferation in a concentration-dependent manner and increases ICAM-1, P-selectin and CXCL-1 expression in IL-1β-induced endothelial cells. These results demonstrate that LM-511 promotes endothelial proliferation and modulates vascular inflammation *in vitro*, providing novel evidence supporting the pro-angiogenic role of LM-511. We are currently establishing the role of LM-511 as a regulator of neutrophil transmigration and angiogenesis *in vitro*

through the use of transmigration assays and established angiogenic assays. Our future aim is to test the hypothesis that LM-511 is a key regulator of BBB repair *in vivo* after experimental stroke by testing the effect of LM-511 deletion on BBB damage, angiogenesis and neurogenesis. Our growing evidence suggests that targeting inflammation and components of the ECM, specifically LM-511, in order to create a cerebral environment that strengthens the NVU and promotes BBB repair is an attractive therapeutic strategy post-stroke.

P I - 5-15
Role of platelets in tissue remodelling and functional recovery after experimental cerebral ischemia
*F. Langhauser[1], P. Kraft[2], C. Kleinschnitz[1]

[1]University Hospital, Neurology, Essen, Germany
[2]Klinikum Main-Spessart, Neurology, Lohr am Main, Germany

Question: Platelets are well characterized mediators of thrombus formation during the acute phase of ischemic stroke. Apart from promoting thrombus formation, platelets also contribute to the local inflammatory response that emerges following stroke. Activated platelets interact with immune cells and this interaction recruits immune cells to the site of vascular injury, thereby contributing to secondary brain damage. Blockade of Glycoprotein Ib was found to ameliorate thrombus formation and invasion of immune cells into the damaged brain, confirming the role of platelets in secondary neuroinflammation. So far, only the impact of platelets on the acute phase of ischemic stroke has been investigated. Therefore we studied the role of platelets in repair mechanisms and long-term recovery following stroke.

Methods: C57BL/6 mice were subjected to 30 minutes of transient middle cerebral artery occlusion (tMCAO). Beginning at day 3 after tMCAO, platelets were depleted using anti-platelet serum. In the following 4 weeks, we performed the adhesive removal test. At day 28 after tMCAO brains were removed. By immunohistochemistry we analysed the extent of thrombus formation within the cerebral microvasculature, the inflammatory response, neurodegeneration and angiogenesis.

Results: Mice without platelets performed significantly better in the adhesive removal test, a sensitive test to assess sensorimotor deficits, compared to non-depleted mice. Platelet depletion reduced microvascular thrombus formation and ameliorated the inflammatory response. At day 28 after tMCAO, significantly fewer CD11b+ macrophages/microglial cells and CD3+ T cells had entered the brain parenchyma of platelet depleted mice when compared with the control group. Degeneration of neurons was significantly ameliorated in platelet-depleted mice, as shown by a higher neuronal density at day 28 after stroke compared to control mice. In the platelet-depleted mice, the vessel density was increased and significantly more vessels were covered by pericytes.

Conclusion: Platelet depletion in the late phase after ischemic stroke improved long-term stroke outcome. Therefor targeted inhibition of platelets not only in acute, but also the chronic phase after ischemic stroke might become a promising strategy to combat ischemic brain damage in the future.

P I - 5-16
Prognostic serum markers for stroke outcome
*B. Clausen[1], M. Grønhøj[2], K. L. Lambertsen[1], Z. Illes[2], B. Finsen[1]

[1]Institute of Molecular Medicine, Department of Neurology, Odense C, Denmark
[2]Odense University Hospital, Odense C, Denmark

Background: Evidence shows that the immune response in the brain and periphery play a key role in early infarct development. In this study, we address the question of a communicatory link between the brain and periphery, mediated by a spillover of inflammatory proteins.

Methods: We analyzed inflammatory proteins (IL-2, IL-4, IL-5, IL-6, IL-6R, IL-1β, TNF, IL-10, IFNγ, CXCL1, IL-12p70 and Hsp70) in two experimental stroke models with a translational comparison to 50 patients suffering from territorial or lacunar ischemic stroke and healthy controls. Correlation analysis was used to determine associations between inflammatory markers, compartments and changes in infarct volume and clinical post-stroke outcomes. The study aim was to understand and uncover potential new blood biomarkers for stroke severity and infarct development.

Results: Our data shows changes in IL-6, IL-1β, TNF, IL-10, CXCL1, IL-12p70 and Hsp70 in the brain, serum or liver from stroke-lesioned mice as well as serum from stroke patients.

Conclusions: Our experimental stroke models mimic the systemic response in stroke patients, identifying two serum proteins as potential prognostic markers in stroke patients.

This study was conducted at the University of Southern Denmark, Odense, Denmark

P I - 5-17
The choroid plexus: a road to the brain for vascular tPA
*V. Zuba[1], S. Martinez de Lizarrondo[1], G. Maxime[1], V. Denis[1], C. Ali[1]

[1]Normandy University, UNICAEN, UMR-S INSERM 1237, Physiopathology and Imaging of Neurological Disorder, Caen, France

Tissue-type plasminogen activator (tPA) is a key player of vascular fluidity. It is also expressed in the central nervous system, where it controls an array of physio-pathological processes (for review, Vivien and Ali, 2012). We previously found that vascular tPA can cross the intact blood-brain-barrier (BBB) by a low-density lipoprotein receptor-related protein 1 (LRP-1)-mediated transcytosis and thus, can also controls brain pathophysiology (Benchenanne *et al.*, 2005). The recent renewed interest in paravascular pathways (Iliff *et al.*, 2013) opens the possibility that the BBB might not be the only door to the brain for tPA. Here, we hypothesized that intravenous tPA can also cross the blood–cerebrospinal fluid barrier (BCSFB), which relies on the choroid plexus (ChP).

First, we coupled recombinant tPA to Alexa555, and performed *in vivo* tracking of its fluorescence in the murine ChP, after intravenous injection. We observed a time-dependent accumulation of fluorescent tPA in choroid plexus epithelial cells of the ChP (CPECs), followed by a decrease in fluorescence with time. Accordingly, tPA activity increased in the CSF following an intravenous injection. This is consistent with tPA passage across the BCSFB. In order to dissect the attendant molecular mechanisms of this passage, we developed a murine model of primary culture of CPECs. We found that CPECs can uptake tPA in a dose- and time-dependent manner. This active passage is prevented at 4°C, as well as by pharmacological (RAP co-administration) or genetic (siRNA) inhibition of LRP-1. Thanks to a pulse-chase approach, we also found that cultured CPECs can release tPA.

In conclusion, we show that tPA translocates from the blood to the CSF via LRP-1. Then, the flow of tPA via paravascular pathways likely contributes to influence brain physiological or pathological conditions.

P I - 5-18

Unravelling the (sub)cellular mechanisms of low frequency electromagnetic stimulation as ischemic stroke therapy

*H. Kemps[1], Y. Dillen[1], L. Perez Font[2], B. Brône[3], R. Lemmens[4], A. Bronckaers[1]

[1]Hasselt University, Biomedical Research Institute, Department of Morphology, Diepenbeek, Belgium
[2]Universidad de Oriente, Centro Nacional de Electromagnetismo Aplicado, Santiago de Cuba, Cuba
[3]Hasselt University, Biomedical Research Institute, Department of Neurophysiology, Diepenbeek, Belgium
[4]KU Leuven, Laboratory of Neurobiology, Center for Brain and Disease Research, VIB, Leuven, Belgium

Background: Neuroprotection for the treatment of acute ischemic stroke has been unsuccessful in clinical practice. We explored low frequency electromagnetic stimulation (LF-EMS) as an emerging safe and non-invasive neuroprotective therapy for the treatment of stroke. Previous data indicated LF-EMS to ameliorate neurological outcome in rats subjected to global cerebral ischemic stroke likely mediated by nitric oxide (NO). However, the mechanism by which NO production is induced remains unknown. Here we studied the effect of LF-EMS on NO production via activation of endothelial nitric oxide synthase (eNOS) in endothelial cells (EC) and the therapeutic response in a focal experimental stroke mice model.

Methods: EC were stimulated with LF-EMS (13.5 mT, 60 Hz) for 20 min or left unstimulated. eNOS phosphorylation (peNOS) was assessed by western blot analysis. A human phospho-kinase array was performed to identify possible upstream targets responsible for eNOS phosphorylation. Phosphorylation of Akt (pAkt) was analyzed via western blot. Distal middle cerebral artery occlusion (dMCAO) was induced in C57BL/6J mice to assess the effect of LF-EMS on infarct volume. dMCAO operated mice were subjected to sham treatment or LF-EMS (13.5 mT, 60 Hz) for 20 min during 4 days. After 7 days, animals were sacrificed and brain slices were stained with TTC.

Results: eNOS activation is regulated by phosphorylation at Ser1177, which is a main phosphorylation site for eNOS activity. LF-EMS significantly increases peNOS at Ser1177 in EC ($p=0.038$, $n=7$). To identify possible upstream targets, a human phospho-kinase array was performed. Based on this array, we investigated whether LF-EMS enhances phosphorylation of Akt, which is an important inducer of peNOS. However, LF-EMS does not significantly induce pAkt in EC ($n=7$). In the experimental stroke model, LF-EMS stimulated dMCAO mice show a trend towards a reduction of 25% in infarct size compared to sham treated dMCAO mice ($p=0.073$, $n=9$).

Conclusion: LF-EMS induces eNOS phosphorylation at Ser1177, which results in enhanced eNOS activity. However, the phosphorylation of the possible upstream kinase Akt is not increased in response to LF-EMS. Other interesting targets identified from the phospho-kinase array will be investigated in future experiments. Data obtained from the dMCAO mouse model suggest that LF-EMS results in smaller infarct volumes compared to sham treated dMCAO mice. Our findings provide more insight into the subcellular mechanisms of LF-EMS, which aid into its clinical translation as an effective therapy for ischemic stroke.

P I - 5-19

Non-erythropoietic EPO derivatives mediate protection in a mice model of brain ischemia/reperfusion injury through the overwhelming effect on the mitochondrial respiration

*I. Mukhina[1,2], P. Pchelin[1,2,3], A. Malashuk[1,2,3], M. Guseva[1,2,3], N. Schelchkova[1,2,3]

[1]Privolzhsky research medical university, Central Research Laboratory, Nizhny Novgorod, Russian Federation
[2]Nizhny Novgorod State University, Department of neurotechnology, Institute of biology and biomedicine, Nizhny Novgorod, Russian Federation
[3],, Russian Federation

Questions: Non-erythrogenic derivative, carbamylated dEPO (CdEPO), have been reported to activate different receptors (homomeric EPO receptor vs. heteromeric receptor consisting of EPO receptor monomer and common β-subunit) in the brain tissue. Due to this property CdEPO is attracting widespread interest for neuroprotection

without erythropoiesis. However, little is known about molecular mechanisms behind CdEPO-mediated neuroprotection. In this regard, the purpose of the study was to study functional bioenergetic characteristics of synaptosome mitochondrial apparatus of cells of the cerebral cortex in the early postischemic period in C57BL/6 mice.

Methods: on the mice model of transient occlusion of the middle cerebral artery the intravenous injection of agonist EPO heterodimer of receptor CdEPO (LLC "Pharmapark", Russia) at a dose of 50 µg/kg was study. Activity of lactate dehydrogenase (LDH), total creatine kinase (CC), lactate and pyruvate, BDNF, GDNF, TGF-β1, HSP70 content, as well as the mitochondrial fraction of LDH, succinate dehydrogenase (SDG) and ATPase were defined. Measurement of mitochondrial oxygen consumption were carried out with the aid of a pyrometer, high-resolution Oroboros Oxygraph-2k (Oroboros Instruments, Austria). To estimate the basal respiration value (in the presence of glutamate and malate breathing substrates but in the absence of ADP), 50 µl of mitochondrial suspension was added to the respirometer chamber (28°C). Oxygen consumption associated with complex I was measured during oxidative phosphorylation after 0.5 M ADP was added to the chamber associated with complex II after 1 M succinate was added.

Results: Heterodimeric erythropoietin receptors agonist (CdEPO) when administered intravenously to mice in the first hours after reperfusion has an overwhelming effect on the mitochondrial respiration of the brain, reducing the intensity of oxygen consumption. Along with this, CdEPO affects the content of some regulatory proteins of the brain (TGF-β1, BDNF, GDNF), leading to a decrease in their levels in brain tissues for 20 days after reperfusion.

Local ischemia causes an increase in the lactate/pyruvate ratio in the blood, with a stable level of LDH activity. The use of CdEPO retains lactate / pyruvate dependence within normal limits. The activity of the mitochondrial fraction of LDH decreased during the experiment in the groups under study but increased after the introduction of the EPOR-βsr agonist. In this group there was an increase in the activity of SDG as an alternative pathway of breathing, against the background of low activity of ATPase.

Conclusions: Non-erythropoietic EPO derivatives mediate protection in a mice model of brain ischemia/reperfusion injury through the overwhelming effect on the mitochondrial respiration, increase in the activity of SDG as an alternative pathway of breathing

P I - 5-20

Contribution of inflammasomes to ischaemic stroke

*E. Lemarchand[1], *J. Barrington[1], A. Chenery[1], G. Coutts[1], C. Hoyle[1], J. E. Allen[1], S. M. Allan[1], D. Brough[1]

[1]University of Manchester, Faculty of Biology, Medicine and Health, Manchester, United Kingdom

Questions: Inflammation plays a key role across the time course of stroke and is known to exacerbate brain damage during the acute phase. Several regulatory molecules are implicated in inflammation, but the most established inflammatory mediator of acute brain injury is the cytokine interleukin-1. Interleukin-1 release from activated immune cells is regulated by the inflammasomes; however, how these inflammasomes are regulated to drive neuronal loss is unknown. Here, we investigate how NLRP3 influences ischaemia brain damage.

Methods: To induce stroke, we used an *in vivo* model of middle cerebral artery thrombosis through topical application of FeCl3 in wild-type and NLRP3-/- mice. Using qPCR and flow cytometry, we investigated the involvement of NLRP3 to damage progression after ischaemic stroke.

Results: We observed elevated levels of NLRP3, IL-1β and Gasdermin D in the infarct 24 hours after stroke. We then measured microglia and infiltrating cells after stroke. We observed infiltrating monocytes and neutrophils into the brain after stroke and that IL-1β was mainly expressed by neutrophils. To determine the role of NLRP3 in stroke, we used MCC950, a NLRP3 specific inhibitor that we injected intra-peritoneally 30 minutes after stroke onset. Lesion volumes were unaltered by MCC950 treatment. This result suggested that NLRP3 inflammasome

activation from infiltrating cells is not involved in IL-1β driving brain damage after stroke. We then injected MCC950 in the cerebral ventricle to investigate the role of NLRP3 in the brain parenchyma. Lesion volumes were again unaltered by MCC950 treatment. To confirm these results, we used mice with gene deletion for NLRP3, and did not observe any change in brain damage.

Conclusions: We found that NLRP3, IL-1β and Gasdermin D present an increase of mRNA level after stroke. However, NLRP3 inhibition or gene deletion does not modify brain damage, suggesting that other inflammasomes are involved during the acute phase of stroke.

P II - 5-21

Role of ADAMTS-4 in the development of Intracranial Aneurysm in mice.

*M. Lepine[1], S. Chagnot[1], C. Ali[1], D. Vivien[1]

[1]INSERM, UMR-S 1237 Physiopathology and imaging of Neurological disorders, Caen, France

Questions: Intracranial aneurysms (IA) are brain vascular malformations that carry a high mortality risk if ruptured. Currently, no reliable non-invasive treatment has been identified, due to a lack of knowledge about mechanisms involved in the formation and rupture of IA. Aneurysms genesis likely involves inflammation and extracellular matrix (ECM) remodeling. ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs-4), a secreted protease, is known to control both inflammation and ECM degradation. We thus hypothesised that ADAMTS-4 could be involved in IA physiopathology.

Methods: We used a mouse model of IA induced by stereotaxic injection of elastase after Angiotensin II infusion to induce hypertension (Labeyrie *et al;* 2017). On a 28 days long magnetic resonance imaging-based follow-up, we compared the formation and rupture of IA in two mouse strains, *wild type* mice and ADAMTS-4 *knocked out* mice. We also performed immunohistochemical phenotyping of IA in both strains.

Results: Our preliminary results, on a small population, suggest that IA is accompanied by a degradation of versican, a component of the ECM in the vascular wall. In addition, characteristic features of inflammation and degradation of smooth muscle cells and basal lamina are also present. This degradation product is the result of proteolytic activity of ADAMTS-4 is with ADAMTS-1 one of the two proteases known to generate the specific versican degradation product found in the IA wall. Interestingly, we found that ADAMTS-4 is expressed by endothelial cells after IA induction. Moreover, the deletion of ADAMTS-4 seems to reduce the average number of IA (~60%).

Conclusions: Our findings suggest an implication of ADAMTS-4 in the formation of IA. We hypothesise that ADAMTS-4 from endothelial origin could be responsible for this destabilization of the vascular wall, by degrading versican. Thus, ADAMTS-4 could be a promising target to prevent the development of IA.

P II - 5-22

Acute cardiac injury following ischemic stroke

*S. Mencl[1], C. Kleinschnitz[1], T. Rassaf[2], U. Hendgen-Cotta[2]

[1]University Hospital Essen, Neurology, Essen, Germany
[2]University Hospital Essen, Cardiology; West German Heart and Vascular Center, Essen, Germany

Background: Cardiac diseases are common post-stroke and are associated with increased morbidity and mortality. One possible mechanism of acute cardiac injury is the neurogenic myocardial damage, where the cerebral injury is disturbing the normal sympathetic and parasympathetic neuronal outflow to the heart leading to cardiac damage including myocardial infarctions. A consequence of an increased sympathetic activity is an exaggerated norepinephrine efflux from cardiac sympathetic nerve terminals into the myocardial interstitium with prolonged opening of the ß1-adrenergic receptor-controlled calcium channels. Abnormal intracellular Ca2+-handling, leads to mitochondrial dysfunction and generation of reactive oxygen species (ROS). The exact mechanism is not completely understood and the major objective of this project is to characterize the molecular phenotype of the neurogenic myocardial damage post-stroke.

Question: Understanding the pathophysiological mechanism of neurogenic myocardial damage

Methods: We analyzed the myocardial damage after left and right sided transient middle cerebral artery occlusion (tMCAO) and left and right sided permanent stroke (photothrombosis; PT). We compared the myocardial damage, e.g. elevated troponin t levels, abnormal echocardiogram, and elevated catecholamine levels in different mouse lines like C57BL/6 J and N, and BALB/C. As a proof of concept we analyzed the

myocardial damage after ß1-adrenergic receptor stimulation using isoproterenol. Furthermore, we induced cerebral stroke in addition with cyclosporine treatment, which is immunosuppressant drug and an inhibitor of the Ca2+ induced mitochondrial damage.

Results: Our data demonstrate acute myocardial damage after tMCAO and PT. Most effect on the myocardial damage had the mouse strain we used for the experiments. Left and right sided or transient and permanent cerebral stroke triggered an elevation of troponin t and catecholamine levels. Following either cerebral stroke or isoproterenol treatment, higher levels of cardiac troponin t and norepinephrine were found in blood and heart samples at distinct time points. The elevated troponin t and norepinephrine levels were less after stroke and cyclosporine treatment.

Conclusion: We found elevated levels of troponin t and catecholamines in the heart after cerebral ischemia or isoproterenol treatment. This myocardial damage was diminished after cyclosporine treatment, indicating that the mitochondrial dysfunction is a part of the pathological mechanism in neurogenic myocardial damage

P II - 5-23

Ischaemia-induced neurofilament affection in various models of experimental stroke

*B. Mages[1,2,3], S. Aleithe[1,3], S. Altmann[1,3], A. Blietz[1,3], B. Nitzsche[4,5], H. Barthel[4], A. K. E. Horn[6], C. Hobusch[2], W. Härtig[3], M. Krüger[2], D. Michalski[1]

[1]University of Leipzig, Department of Neurology, Leipzig, Germany
[2]University of Leipzig, Institute of Anatomy, Leipzig, Germany
[3]University of Leipzig, Paul Flechsig Institute of Brain Research, Leipzig, Germany
[4]University of Leipzig, Department of Nuclear Medicine, Leipzig, Germany
[5]University of Leipzig, Institute of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Leipzig, Germany
[6]Ludwig-Maximilians-University, Institute of Anatomy and Cell Biology I and German Center for Vertigo and Balance Disorders, Munich, Germany

Question: While neurofilaments are known to be involved in maintaining cellular integrity, their role during stroke progression remains poorly understood. Remarkably, circulating neurofilaments were found to reflect the course and severity of cerebral ischaemia. This allows for the hypothesis that an affection of the neurofilament network is a critical hallmark towards the transition from temporary to long-lasting tissue damage. Therefore, neurofilaments might be considered as a so far neglected target for novel neuroprotective approaches in stroke.

Methods: Using three different models of middle cerebral artery occlusion (MCAO) in mice, rats and sheep added by human autoptic stroke tissue, we systematically addressed ischaemia-induced alterations of the four neurofilament components. Neurofilament light (NF-L), medium (NF-M) and heavy (NF-H) chains as well as α-internexin (INA) were analysed using multiple immunofluorescence labelling, Western Blot analyses and qRT-PCR.

Results: Ischaemia induced a consistently reduced immunofluorescence signal for NF-M and INA in the applied animal models and in human stroke tissue. Further, a distinct demarcation of the neocortical ischaemic border was indicated by NF-L immunolabelling. Remarkably, a histochemical affection of neurofilaments also extended to areas assumed to reflect the ischaemic penumbra as indicated by an upregulation of the heat shock protein 70 in neurons. Biochemical analysis confirmed a significant reduction of NF-L and INA in the ischemia-affected cortex after MCAO in mice. Notably, at the mRNA level a reduced expression of NF-L- and NF-H-related genes was observed in neocortical areas, while the striatal gene expression of INA appeared upregulated.

Conclusion: In a multi-methodological approach, neurofilaments were confirmed as highly sensitive structures in ischaemic brain conditions. As the described alterations include potentially salvageable tissue of the ischaemic penumbra, a pharmacological modulation of the neurofilament network appears conceivable for upcoming therapeutic strategies.

P II - 5-24

Understanding the role of circulating extracellular vesicles in systemic response to ischemic stroke

*L. Rosa-Fernandes[1], M. Møller-Nielsen[1], M. R Larsen[1], B. Clausen[1], K. Lambertsen[1]

[1]University of Southern Denmark, Odense, Denmark

Background: Ischemic stroke is the result of a vessel blockage, impairing the blood supply within a brain region. The lack of blood supply, and in consequence of the lack of nutrients, induces neuron depolarization leading to necrotic cell death in the ischemic core. Whereas, the surrounding region, known as penumbra, is characterized by the presence of apoptotic cells. Besides the local events induced by the vessel occlusion, a systemic reaction is also triggered by the activation of acute-phase response (APR), which is mediated by the release of acute-phase proteins (APPs) in the blood stream. APPs can be detected in the circulation even before signs of inflammatory response in the brain. However, the signalling mechanisms promoting such systemic response remain unclear.

In the past decade, extracellular vesicles (EVs) have been recognized as molecular messengers for intercellular communication and have prompted interest due to potential function in prognosis and therapeutic guidance in pathological conditions. Since the protein cargo of EVs is disease-dependent, this work used mass spectrometry-based proteomics to investigate the role of circulating microvesicles and exosomes after brain injury as the promotor of a systemic response.

Methods: Plasma from mice subjected or not to permanent middle cerebral artery occlusion (p-MCAo) was collected after 12h. Plasma microvesicles (MVs) and exosomes (Exs) were isolated by differential ultracentrifugation. After protein extraction and tryptic digestion, chemical labeling strategy (iTRAQ) was adopted, followed by HILIC fractionation and nLC-MS/MS analysis. This approach made possible the assessment of circulating EVs cargo proteins regulation after ischemic stroke.

Results: Using this strategy, we were able to identify a total of 940 MVs and 794 Exs proteins. Relative quantification comparison of proteins identified with two or more peptides showed 131 MVs and 128 Exs regulated proteins between mice subjected to p-MCAo and respective sham (Limma test q-value <0,05). Among those, 96 and 103 were up-regulated while 35 and 24 were downregulated in microvesicles and exosomes, respectively.

Immune system process, oxidoreductase activity and cellular response to IL-4 were among the over represented biological processes (pValue <0.05, B-H FRD 5%). Pathway prediction analysis showed "Inflammatory response" among overrepresented annotations.

Conclusions: The induction of ischemic stroke in mice is able to modify in the protein cargo content of blood stream circulating extracellular vesicles after 12h. Microvesicles and exosomes bear different protein content.

Keywords: Ischemic stroke; circulating extracellular vesicles; systemic response; proteomics

P II - 5-25

The role of systemic inflammation on cortical blood flow during the acute phase after experimental stroke

*O. Saleh[1], S. Allan[1], I. Schiessl[1]

[1]University of Manchester, Faculty of Biology, Medicine and Health, Manchester, United Kingdom

Restoring cerebral blood flow after ischaemic stroke is critical for improving patient outcome and reducing neuronal injury. Clinically this can be achieved through pharmacological recombinant tissue plasminogen activator (tPA). Reperfusion therapy is only suitable for a small percentage of stroke patients and there is often incomplete reperfusion. Studying the dynamics of cerebral blood flow is essential in order to improve our understanding about the mechanisms underlying the no-reflow phenomenon that prevents the brain from

receiving adequate blood perfusion. Recent evidence has demonstrated that systemic inflammation is an important contributing factor in the no-reflow phenomenon and interleukin (IL)-1 is a key cytokine known to induce systemic inflammation. Hence, we hypothesise that systemic inflammation induced by IL-1 will impair blood flow and we will investigate the early change of blood flow after experimental stroke. We will use the middle cerebral artery occlusion (MCAO) model in C57 mice using a doccol filament to induce stroke. We then image blood flow changes on the cortical surface using laser speckle spectroscopy. We intraperitoneally (i.p.) inject 20 ng/kg of mouse recombinant IL-1β 30 min prior to the MCAO to investigate the effect of systemic inflammation on early changes of cerebral blood flow after stroke. Post-mortem tissue damage will be assessed using immunohistochemistry including cresyl violet (nissl) staining for neuronal cell damage, IgG for blood-brain barrier breakdown, Iba-1-positive activated microglia identification, GFAP-positive activated astrocyte staining, CD41-positive platelet cell identification and staining for IL-1α. These findings will further our understanding of the role of acute systemic inflammation in the early stages of blood reperfusion following ischaemic injury

P II- 5-26

Lesional and perilesional tissue characterisation by automated image processing in a novel gyrencephalic animal model of peracute intracerebral haemorrhage

*B. Nitzsche[1,2,3], A. H. Hainsworth[4], L. R. Brigdes[4], M. Zille[5], D. Lobsien[6,5], H. Barthel[1,5], D. D. McLeod[7], F. Gräßer[8], A. K. Schatzl[9], S. Pietsch[3], A. Y. Dreyer[9], J. Boltze[3], F. Ferrara[10]

[1]University of Leipzig, Nuclearmedizin, Leipzig, Germany
[2]University of Maastricht, Pharmacology and Personalised Medicine, Maastricht, Netherlands
[3]University of Lübeck, Institute for Medical and Marine Research, Lübeck, Germany
[4]St George's University of London, Molecular and Clinical Sciences Research Institute, London, United Kingdom
[5]University of Lübeck, Institute for Experimental and Clinical Pharmacology and Toxicology, Lübeck, Germany
[6]University of Leipzig, Leipzig, Germany
[7]The University of Newcastle, Faculty of Health and Medicine, Callaghan, Australia
[8]Technical University of Dresden, Institute of Biomedical Engineering, Faculty of Electrical and Computer Engineering, Dresden, Germany
[9]Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany
[10]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Questions: Intracerebral haemorrhage (ICH) is a highly relevant stroke subtype. However, preclinical research on ICH is somewhat limited due to a lack of translational animal models. Large gyrencephalic animal models are useful to (i) comparatively investigate key pathophysiological parameters to human ICH, (ii) apply complex imaging-based investigations using clinical equipment and, (iii) utilise reproducible and objective image analyses procedures. In accordance with the HEADS recommendations, we established an ICH model in sheep, and developed an advanced neuroimage processing pipeline for automatic brain tissue and hemorrhagic lesion determination in this model.

Methods: For ICH modelling, 14 adult sheep (5/9 male/female) were assigned to stereotactic autologous blood injection into cerebral white matter under physiological monitoring after baseline MR imaging procedure (1.5T Phillips MRI, sequences: T1w, T2w TSE, T2*). T1w, T2w TSE, T2*, diffusion and perfusion weighted MR imaging was performed 6 hours after ICH followed by sacrificing. Image analyses include automatic brain tissue and lesion segmentation, quantitative volumetry and voxel-based-morphometry (VBM) before and after ICH. Subsequent neuropathological investigation including immunohistological staining was conducted.

Results: Controlled, stereotactic application of autologous blood caused a space-occupying intracerebral haematoma of moderate extent, predominantly affecting white matter at 6 hours post-injection. Neuroimage post-processing using lesion probability maps enabled automatic tissue and lesion classification. VBM showed the compression of the ipsilateral lateral ventricle ($p = 0.003$) and a bilateral dilation of the ventricle of the olfactory bulb ($p < 0.001$). Neuropathological and immunohistological investigation revealed perilesional vacuolation, axonal damage, and perivascular blood as seen after human ICH.

Conclusion: Peri-haematomal characteristics after modelled ICH in sheep are comparable to the findings reported in human beings. The model and imaging platform reflects key aspects of human ICH, and enables future translational research on haematoma expansion/evacuation, white matter changes, haematoma evacuation, and other aspects.

P II - 5-27

Non-pulsed sinusoidal electromagnetic field rescues animals from severe stroke through induction of angiogenesis and nitric oxide.

*A. Bronckaers[1], L. P. Font[2], H. Kemps[1], M. M. Cardonne[2], R. Meesen[1], I. Lambrichts[1], F. G. González[2], J. M. Rigo[1], B. Brone[1]

[1]Hasselt University, BIOMED, Diepenbeek, Belgium
[2]Universidad de Oriente, Centro Nacional de Electromagnetismo Aplicado, Santiago de Cuba, Cuba

Question: Despite the high prevalence and devastating outcome of cerebral ischemic stroke, only a few treatment options exist. Extremely low frequency magnetic fields (ELF-MF), which are defined by a magnetic flux below 20mT in a frequency range of 1 to 300 Hz, have been repeatedly reported to have beneficial effects in preclinical models of various neurological disorders. They have been applied to treat delayed union bone fractures, wounds, knee osteoarthritis and edema. Previous reports also indicated that they are able to induce angiogenesis, i.e. blood vessel formation. As stimulation of blood vessel formation has a potential as an effective treatment for stroke, we investigated whether and by which mechanisms 'Non-Pulsed Sinusoidal Electromagnetic Field (NP-SEMF)' stimulation has beneficial effects in cerebral ischemia.

Methods: Ischemic injury was induced by occlusion of both common carotid arteries in rats. NP-SEMF treatment existed of 20 minutes exposure at either 10 or 60 Hz and 13.5 mT for 4 days. The survival, neurological score and amount of affected tissue were measured, as well as the number of blood vessels 7 days after surgery. The effect of NP-SEMF on endothelial cell behavior as well as nitric oxide (NO) production was assessed *in vitro*.

Results: In the rat model of cerebral ischemia, NP-SEMF dramatically increased the survival and significantly improved the neurological scores 7 days after surgery (n=13/group, $p<0.01$). The amount of necrotic brain tissue was also significantly reduced. The number of blood vessels in the hippocampus of animals treated with 60Hz NP-SEMF was 2.3-times higher compared to untreated rats (19.4±4.7 versus 8.5±4.1 in control). In endothelial cells *in vitro* NP-SEMF enhanced important steps of the angiogenic cascade such as proliferation, migration and tube formation. NO concentration was significantly elevated in endothelial cells treated with 60Hz, 24h after application (3.9±1.3 µM in 60 Hz vs. undetectible values for control cells). In addition, NO was shown to be an essential molecule in NP-SEMF induced endothelial cell migration, as inhibitors of nitric oxide synthase inhibited this process. The importance of NO as a key signaling molecule activated by NP-SEMF was also proven *in vivo* by the fact that blocking NO with the nitric oxide synthase inhibitor L-NAME significantly abrogated the beneficial effects of NP-SEMF on survival, neurological score and affected brain area in the rat stroke model(n=6/group, $p<0.01$).

Conclusion: Our results indicate for the first time that NP-SEMF improves the survival and neurological outcome after cerebral ischemia in rats and prove an essential causal link with angiogenesis through endothelial NO production.

P II - 5-28

Microglial derived interleukin 1 is essential for myeloid recruitment and has neuroprotective actions after intracerebral haemorrhage

*J. Barrington[1], J. Rivers-Auty[1], A. Parry-Jones[1], D. Brough[1], S. Allan[1]

[1]University of Manchester, Faculty of Biology, Medicine & Health, Manchester, United Kingdom

Question: Intracerebral haemorrhage (ICH) is a devastating subtype of stroke that causes death or disability in two thirds of patients. Despite being 4-fold less common than ischaemic stroke, ICH causes an equivalent loss in disability-adjusted life-years. There is therefore a great unmet clinical need for ICH therapies. Some inflammatory processes are known drivers of neurological injury in ICH yet the role of the immune response remains to be fully elucidated. The pro-inflammatory interleukin-1 (IL-1) axis contributes to deleterious processes in many types of acute brain injury. However, it is currently unknown what role IL-1 plays in ICH. Here we set out to investigate IL-1 signalling and its downstream consequences after ICH.

Methods: To assess inflammatory responses to ICH we utilised a collagenase injection murine model. To identify the cellular distribution of IL-1, brains were processed for histology or single cells were isolated for flow cytometry or protein analysis. Histological findings were then validated by immunostaining peri-haematomal clinical samples, obtained from the Edinburgh Brain Bank, alongside age-matched region-matched controls. To evaluate IL-1 dependent processes, mice were given a 10 µg intrastriatal injection of IL-1 receptor antagonist (IL-1ra) prior to ICH, followed by six 100 mg/kg subcutaneous doses over three days. A powered randomised blinded controlled investigation was designed and implemented to assess effects of IL-1 inhibition on rotarod performance as readout for neuromotor dysfunction.

Results: In both human and murine ICH we discovered early IL-1 production in perihaematomal microglia that precedes large myeloid recruitment to the murine brain. Moreover, direct inhibition of IL-1 signalling through administration of IL-1 receptor antagonist (IL-1ra) completely abolished innate immune recruitment 24h after ICH. Counterintuitively however, this acute inhibition of IL-1 signalling worsened neuromotor injury in a mouse model of ICH.

Conclusion: We have therefore shown that IL-1 is the key driver of inflammation in ICH and this is initiated by brain resident microglia. However, in contrast to other types of acute brain injury, microglia derived IL-1 has a protective effect after ICH, challenging the notion that inflammation exacerbates injury during acute time-windows. Ongoing studies look to elucidate the mechanism of IL-1 neuroprotection in order to highlight modifiable pathways for future therapeutics.

P II - 5-29

Hypoxia-induced changes to endoplasmic reticulum calcium and proteostasis

*K. Trychta[1,2], J. Anttila[2], X. Yan[1], K. J. Wu[3], Y. Wang[3], M. Henderson[1], M. Airavaara[2], B. Harvey[1]

[1]National Institute on Drug Abuse, Molecular Mechanisms of Cellular Stress and Inflammation, Baltimore, United States
[2]University of Helsinki, Institute of Biotechnology, Helsinki, Finland
[3]National Health Research Institutes, Zhunan, Taiwan

Question: Calcium within the endoplasmic reticulum (ER) lumen is maintained at a much higher concentration than calcium in the cytoplasm. This calcium gradient is important for many cellular functions, including protein trafficking, lipid metabolism, and signaling pathways. Depletion of ER calcium has been linked to multiple disorders of the central and peripheral nervous systems, including stroke. The purpose of the current study is to examine how ER calcium dysregulation contributes to stroke pathogenesis.

Methods: Using a previously described *Gaussia* luciferase secreted ER calcium-modulated protein (GLuc-SERCaMP) we monitored ER calcium depletion in models of ischemia. Ischemia was induced via oxygen and

glucose deprivation *in vitro* and middle cerebral artery occlusion (MCAo) *in vivo.* For *in vivo* studies, cerebrospinal fluid (CSF) was collected at different time points post-stroke to monitor SERCaMP secretion. We also developed a series of GLuc-based SERCaMP reporters representing ~80 ER resident proteins to track changes in the ER proteome in response to hypoxia.

Results: We demonstrate here that oxygen and glucose deprivation *in vitro* increased ER calcium depletion and decreased cell viability. We observed a widespread shift in GLuc-SERCaMP reporters following ischemia, which suggests ER resident proteins are redistributed into the extracellular environment following ischemia. We have defined this departure of proteins from the ER in response to a pathophysiological condition "ER exodosis." Dantrolene, a ryanodine receptor (RyR) antagonist that attenuated ER calcium depletion and increased cell viability, also decreased SERCaMP release. Furthermore, rats injected with AAV vectors expressing GLuc-SERCaMP in the cortex showed evidence of ER calcium depletion following occlusion of the middle cerebral artery, and a dantrolene treatment reduced the infarct volume.

Conclusions: Collectively, our data supports a model in which ischemic injury causes ER calcium depletion and ER exodosis. These effects can be attenuated by stabilizing ER calcium and preventing loss of ER resident proteins.

P II - 5-30
Accumulation of β-amyloid variants in correlation with post-stroke neuroinflammation pathology, at cortical stroke model in rats with distal middle cerebral artery occlusion
*S. Er[1], J. E. Anttila[1], A. Domanskyi[1], M. T. Airavaara[1]

[1]University of Helsinki, Institute of Biotechnology, Helsinki, Finland

Ischemic stroke is a major cause of disability among all age groups, most commonly for the people over 60 years old. Therapeutic strategies for post-stroke recovery are needed to be improved for the achievement of better neuroprotection, which can improve neurologic dysfunctions in patients. In order to develop new treatments, more studies should be conducted on the post-ischemic pathologies. We have focused on neuroinflammation and accumulation of β-amyloid precursor protein (APP) that is processed into β-amyloid (Aβ). This hydrophobic, self-aggregating peptide is the hallmark pathology of Alzheimer"s disease. Elevated rates of APP and accumulation of Aβ at the ischemic regions are also documented for post-ischemic models, however its role in this progress is understudied. The activation of astrocyte and microglia at these regions suggest there might be a link between the accumulation of β-amyloid variants and neuroinflammation at post-stroke thalamus. We have previously characterized the temporal progression of microglia/macrophage activation following cortical ischemic injury in rat between days 2-112. Distal middle cerebral artery occlusion together with common carotid artery occlusion for 90 min was used as the cortical stroke model in rats. The results demonstrated that the peak activation of phagocytic cells occurs in cortex occurs at 7 d post-stroke ($n=4$, $p<0.05$), and chronic expression of phagocytic cells is associated with neuronal loss in the thalamus. As a continuation of the previous study, we investigated the time-dependant accumulation of β-amyloid variants in post-stroke rat cortex, and its possible association with the neuroinflammation pathology, and these results will be presented in the poster.

P II - 5-31
Role of FXII in long-term stroke recovery
*R. D. Steubing[1], F. Langhauser[1], C. Kleinschnitz[1]

[1]Universitätsklinikum Essen, Neurologie, Essen, Germany

Question: Inflammation, edema formation and thrombosis are prominent features of ischemic stroke. Coagulation factor XII (FXII) activates the intrinsic coagulation pathway and the Kallikrein-Kinin-System and pushes thrombosis and inflammation. Since it is already known that inhibition of FXII is protective in the acute phase of stroke, little is known about its role during late-stage neuroregeneration. Therefore we performed a

study on the role of FXII-mediated thrombo-inflammation for post-stroke regeneration with blocking of activated FXII using the specific FXIIa inhibitor rHA-Infestin-4.

Methods: To address how a FXII inhibition affects neuroregeneration in the chronic phase after stroke, we inhibited FXIIa with rHA-Infestin-4 in wild-type mice beginning at day 3 until day 10 after 30 minutes of transient middle cerebral artery occlusion (tMCAO). At this point, infarct maturation was already completed and we matched all treatment groups so there were no differences in infarct volumes prior to FXIIa inhibition. In the following 4 weeks, we performed functional tests, to analyse possible deficits. The local inflammatory response was determined by immunohistochemistry.

Results: Interestingly, mice without FXIIa activity performed significantly better in the adhesive removal test, a sensitive test to assess sensorimotor deficits, compared to control mice. Having a look at neuroinflammation, we found that at day 28 after tMCAO significantly fewer CD11b+ macrophages/microglial cells, neutrophils and CD3+ T cells had entered the brain parenchyma of FXIIa inhibited mice when compared with the control group ($p<0,001$).

Conclusions: FXII is critically involved in the pathophysiology of ischemic stroke via the activation of inflammatory and thrombotic circuits, not only in the acute, but also in the chronic phase after the ischemic insult. We have demonstrated that the selective FXIIa inhibitor rHA-Infestin-4 protects mice from neuroinflammatory events und sensorimotor deficits after ischemic stroke. These findings indicate that blocking of FXIIa could become a promising and safe option to combat this devastating neurological disease.

P II - 5-32

Interleukin 1α is profoundly neuroprotective and neurorestorative following experimental ischemic stroke

K. Salmeron[1], M. Maniskas[1], *D. Edwards[1], R. Wong[2], A. Trout[1], S. Hamilton[1], J. Fraser[1,3], E. Pinteaux[2], *G. Bix[4]

[1]University of Kentucky, Neuroscience, Lexington, United States
[2]University of Manchester, Manchester, United Kingdom
[3]University of Kentucky, Neurosurgery, Lexington, Germany
[4]University of Kentucky, Neurology, Lexington, United States

Stroke remains a leading cause of death and disability worldwide despite recent breakthroughs in efforts to recanalize occluded vessels. 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. Inflammation after stroke 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.

Question: IL-1a could represent a novel neuroprotective and neurorestorative therapeutic target in ischemic stroke. 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 angiogenic LG3 fragment.

Methods: Young male mice were subjected to middle cerebral artery occlusion for 1 hour. For neuroprotection experiments, 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. For neurorepair experiments, animals were treated with IV IL-1a on post stroke day three. Infarct volume was quantified using cresyl violet staining, microglial activation was assessed through CD11b immunostains, and apoptotic cell death was evaluated via TUNEL staining. Behavioral deficit and recovery was scored using an 11-point score. IL-1a"s

neuroprotective potential was also tested *in vitro* via OGD of primary cortical neurons, while its potential mechanism of action was tested using brain endothelial cells (BEC).

Results: 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 microglial activation than vehicle controls. We also found that IL-1a is neuroprotective from OGD injury on primary neurons *in vitro.* Delayed post-stroke IV IL-1a administration enhances functional recovery, and also promotes brain angiogenesis. Finally, we found that treating BECs with IL-1a significantly increased perlecan and the LG3-generating protease cathepsin B mRNA.

Conclusion: IL-1a is neuroprotective and neuroreparative in experimental stroke with side effects (IA), and may do so via the generation of perlecan LG3.

P II - 5-33
Neuroprotective therapy both for acute ischemic stroke and ALS
*K. Abe[1], T. Yamashita[1], R. Morihara[1], Y. Nakano[1], Y. Ohta[1], J. Shang[1]

[1]Okayama University, Neurology, Okayama, Japan

Neuroprotection is essential for potential therapy not only in acute stage of stroke but also in chronic progressive neurodegenerative diseases such as ALS, Parkinson"s disease (PD), and Alzheimer"s disease (AD). Free radical scavenger can be such a neuroprotective candidate with inhibiting death signals and potentiating survival signals under cerebral ischemia and even neurodegenerative cellular processes. Edaravone is one such free radical scavenger, which is the first clinical drug for neuroprotection in the world and has been used from 2001 in most ischemic stroke patients in Japan (1). Edaravone scavenges hydroxyl radicals both in hydrophilic and hydrophobic conditions, and is especially useful in thrombolytic therapy with tissue plasminogen activator (tPA) for reducing hemorrhagic transformation. An intensive Edaravone therapy for 3 days now showed a favorite recovery in 3 European countries (2).

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease caused by selective death of motor neurons. Among our own 390 ALS patients, 4.1% show familial ALS (FALS), in which 50% is associated with missense mutations of SOD1, 25% were TDP43 and FUS mutations, and 6.3% is an optineurin mutation. Although the underlying mechanism of ALS has not yet been fully clarified, several reports have implicated the involvement of oxidative stress under selective death of motor neurons in both ALS patients and animal models (Warita et al., 2001; Barnham et al., 2004; Abe K, 2007; Miyazaki et al., 2009; Barber and Shaw, 2010).

A recent multicenter prospective double-blind placebo-control clinical trial with edaravone for ALS patients conducted in Japan showed a positive effect for delaying the clinical score (ALSFRS-R) during the course of examination (24 weeks). Serious or critical adverse effect was not noted in this clinical trial. Of particular was that this clinical benefit of edaravone was shown as an add-on therapy after anti-glutamatergic riluzole (3). These data strongly suggest a potential underlying mechanism of oxidative stress both in acute ischemic stroke and in ALS by a free radical scavenger. Edaravone is approved for ALS on 2015 in Japan, 2016 in Korea, and 2017 in USA, and currently in consideration for EU countries.

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P II - 5-34

Reduced tonic inhibition after stroke promotes motor perfomance and epileptic seizures

*C. Frahm[1], O. W. Witte[1], N. Jänisch[1], C. Hübner[1], L. Liebmann[1], M. Günther[1]

[1]Universitätsklinikum Jena, Jena, Germany

Stroke survivors often recover from motor deficits, either spontaneously or with the support of rehabilitative training. Since tonic GABAergic inhibition controls network excitability, it may be involved in recovery. Middle cerebral artery occlusion in rodents reduces tonic GABAergic inhibition in the structurally intact motor cortex (M1). Transcript and protein abundance of the extrasynaptic GABAA-receptor complex α4β3δ are concurrently reduced (δ-GABAARs). In vivo and in vitro analyses show that stroke-induced glutamate release activates NMDA receptors, thereby reducing KCC2 transporters and down-regulates δ-GABAARs. Functionally, this is associated with improved motor performance on the RotaRod, a test in which mice are forced to move in a similar manner to rehabilitative training sessions. As an adverse side effect, decreased tonic inhibition facilitates post-stroke epileptic seizures. Our data imply that early and sometimes surprisingly fast recovery following stroke is supported by homeostatic, endogenous plasticity of extrasynaptic GABAA receptors.

P II - 5-35

A 3D Labeling Approach In Solvent-Cleared Brains To Analyse Axonal Projection Profiles After Cortical Stroke

*K. Christof[1], P. Gabor[1,2]

[1]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany
[2]University Hospital Bonn, Department of Neurology, Bonn, Germany

Question: Stroke is a major cause of death and disability worldwide that warrants development of effective treatments to ameliorate insult burden. In this project, we aim to establish a labeling approach throughout the mouse cortex to analyse axonal projection profiles.

Methods: Male C57Bl/6N mice receive an injection of Rose Bengal (RB) into the tail vein. The left cortical forelimb motor cortex (M1) is exposed to laser light of 561nm. Upon irradiation, RB produces singlet oxygen, thereby initiating the formation of thrombi in small cortical vessels (photothrombotic infarct). Three weeks after stroke, tracer virus and dye molecules are injected: i) AAV9.hSyn.eGFP directly adjacent to the infarct to anterogradely label axon bundles, and, ii) cholera toxin b (CTb-AF594) into the premotor cortex (PMC) to back-label cells that project to this area. Two weeks after tracer injections, mice are perfused and brains subjected to organic solvent-based clearing (BABB method) followed by imaging on a 2-photon microscope. Furthermore, mice are assessed on an elevated grid walk to monitor motor deficits.

Results: Preliminary results of our retrograde labeling study indicate that there is a significant reduction of CTb-positive somata (cells that project to the PMC) anterior, medial and lateral to the stroke, compared to sham controls. In contrast, no significant reduction of cells is observed in the PMC in stroke animals (N=15 for each group, two-tailed t-test).

The anterograde labeling approach with AAV9.hSyn.eGFP is currently ongoing in both sham and stroke animals, but preliminary data indicate a change in cortical projection profiles in ischemic mice in both premotor and somatosensory cortical areas as compared to sham mice as analyzed by 3D cluster analysis.

In addition, mice perform significantly worse on an elevated grid walk with their contralateral fore- and hindpaws (increased number of paw misplacements) after stroke as compared to both ipsilateral paws and sham controls

(N=15 per condition, Repeated Measures ANOVA). We will present preliminary data on how different drugs affect axon projections and motor outcome.

Conclusions: In summary, our model and technique allow for comprehensive and in-depth analyses of long-range projections after stroke and their modulation for systemic or local pharmacotherapies aimed at promoting axon sprouting in subsequent translational trials.

P II - 5-36
Analyzing post-stroke recovery by widefield cortical calcium imaging
*J. Cramer[1], B. Gesierich[1], S. Roth[1], M. Düring[1], A. Liesz[1]

[1]Institute for stroke and dementia research, LMU, Munich, Germany

Questions: Brain networks are changing after cerebral infarction and reestablishment of cortical network function is a hallmark of post-stroke regeneration. Previous studies using fMRI in humans have advanced our knowledge about neuronal plasticity and recovery after stroke. In a reverse translational approach, we aimed to establish analytical strategies similar to those used in fMRI research in mouse in vivo imaging. We established the wide field in vivo calcium imaging of both brain hemispheres in reporter mice expressing a calcium sensor in excitatory neurons. This novel tool enables tracking of cortical network alterations during post-stroke recovery.

Methods: We used heterozygous Thy1-GCamP6s mice of 14 weeks age. After applying a chronical cortical window with approximately 8 mm diameter, we performed in vivo widefield calcium imaging. In order to identify functional areas in the brain of individual mice we used an independent component analysis (ICA). Subsequently, we induced photothrombotic lesion in the respective motor cortex. In an additional set of animals we used the sensory cortex to induce a lesion, identified by analyzing activity recorded during hindlimb sensory stimulation. We followed the regeneration process, in both groups with motor and sensory cortex lesions respectively, by recording resting state data over 4 minutes up to 2 months after stroke. For the sensory lesion, we additionally acquired stimulation data of both affected and unaffected limb. All data was spatially co-registered to ensure comparability between individual mice and timepoints. To investigate changes in inter- and intrahemispheric cortical functional connectivity levels, we calculated Pearson"s correlation on signal time courses of functional brain areas, determined by ICA. Motor rehabilitation was assessed using a multi-parameter neuroscore and the beam walk test.

Results: We found strong interhemispheric functional connectivity, especially in homotypic areas, at baseline. We observed a severe loss in interhemispheric but a slight increase in contralateral intrahemispheric connectivity in the acute phase after stroke. In chronic phase the interhemispheric correlation of calcium signals increased but did not fully recover to baseline levels up to day 56 post lesion. In line with this, behaviour tests showed prominent motor deficits within the first days after stroke. These deficits improve within the 2 month follow up but did not restore fully to baseline levels.

Conclusions: We introduce a novel paradigm of in vivo widefield calcium imaging as a tool to extend translational methods for investigating brain network recovery. After stroke, we suggest the contralateral hemisphere to transiently compensate neurological function. Long-term restoration of functional connectivity and motor rehabilitation correlate which emphasizes the importance of further investigation of brain networks to identify mechanism of recovery and adequate timepoints for interventions.

P II - 5-37

Silent cerebral microbleeds induce long-term cognitive decline in the non-diseased mouse.

*P. Maud[1], O. Thavarak[1], B. Vincent[1]

[1]University of Lille-Inserm-CHU Lille, U1171-Degenerative and vascular cognitive disorders, Lille, France

Introduction: Cerebral microbleeds (CMBs) are suspected to precipitate cognitive decline in patients suffering from cerebrovascular disease. Whether CMBs are able to impair cognition by themselves or through the promotion of intracerebral haemorrhage (ICH) is a critical issue for anticoagulated subjects.

Methods: We induced a disseminated pattern of ~20 CMBs in the Sylvian territory of the brains of wild-type mice through intra-carotid injection of methylated cyclodextrin nanoparticles. After seven days of onset, the CMB burden did not impair locomotor (actimetry) nor cognitive functions (novel object recognition and spontaneous alternance tests), thus remaining silent. In parallel, clinical-based doses of several oral anticoagulants were administered three days before and during the onset.

Results: Whereas the vitamin K-antagonist warfarin provoked ICH and increased mortality (+45%),direct oral anticoagulants (apixaban, rivaroxaban or dabigatran) did not, but enhanced the CMB burden through a greater number of intermediate sized microbleeds (+80% to +180%).While these enhanced CMB burdens remained silent seven days after onset, they increased mortality (+11% to 58%) along the following year without any progression or transformation into ICH despite the cessation of anticoagulation treatment. Neuropathological examination showed a dynamic profile of CMBs including intact or lyzed erythrocytes, as well as iron-positive haemosiderin deposits. Surprisingly, both vehicle- and anticoagulant-treated survivors exhibited progressive cognitive decline (-95% to -135%, novel object recognition test only), with a precipitation of the age-related reduction of locomotor function (-35% to -50%), from 9 months to 12 months, with no statistical difference between groups.

Conclusion: Cerebral microbleeds are able to induce cognitive decline in mouse in a non-diseased setting, and without spontaneous or anticoagulant-induced haemorrhagic tranformation.

P II - 5-38

Long-Term Dynamics after Transient Cerebral Artery Occlusion(tMCAO) in Mice

*R. I. Kestner[1], R. Vutukuri[2], L. Hansen[2], A. Lourbopoulos[3], S. Günther[4], K. Devraj[2], R. Brunkhorst[1], W. Pfeilschifter[1]

[1]Goethe University, Neurology, Frankfurt, Germany
[2]Pharmazentrum Goethe University Frankfurt, Institute for General Pharmacology and Toxicology, Frankfurt, Germany
[3]University of Munich Medical Center, Institute for Stroke and Dementia Research, Munich, Germany
[4]Max-Planck-Institute for Heart and Lung Research, Department of Cardiac Development and Remodeling, Bad Nauheim, Germany

The challenge of translating successful preclinical into clinical advances in stroke research is partly due to limitations of the established in-vivo models. TMCAO in mice models closely the I/R injury of patients with acute large vessel occlusion and recanalization. Due to high mortality of this model long-term observations were hitherto impossible. We used a new care protocol comprising close clinical monitoring and individual care which increased the survival rate > 70% to get first representative insights into the dynamics of post-reperfusion inflammation and regeneration. Special focus was on the contribution of S1P as it is known to be a crucial regulator of barrier integrity and immune cell migration. C57Bl/6 mice underwent tMCAO vs. sham operation and were supported according to a recently established protocol. Brains were removed after 24 hours or 7 days respectively, hemispheres were separated and processed for microvessel and subsequent mRNA isolation for whole transcriptome mRNA-sequencing.

After 24 hours, intracellular S1P production by SphK1 and secretion by Spns2 is highly upregulated while expression of S1P-Lyases is decreased, which normalizes during tissue remodeling. The overall dynamic changes from an inflammatory condition, dominated by IL1-beta and IL6, to a highly proliferating condition determined by massive increase of KI67 paralleled by upregulation of microglial and ECM markers. Those results are currently being validated by quantitative PCR in dissociated cell fractions of the neurovascular unit and by MELC staining to achieve a spatio-temporal model of the acute and subacute processes after I/R injury in brain vessels.

Intense post-stroke care allows first representative insights into the long-term dynamics of cerebral I/R injury and indicative testing of future therapeutic strategies. In analogy to stroke-unit monitoring and support of patients, this approach aligns the tMCAO model closer to the clinical situation and contributes to successful translational stroke research.

P II - 5-39
The impact of collaterals on reperfusion in stroke
*M. El Amki[1], N. Binder[1], M. Wyss[1], T. Najimi[1], B. Weber[1], S. Wegener[1]

[1]Universitätsspital Zürich, Department of Neurology, Zurich, Switzerland

Aim: Despite improvements in acute recanalization treatments, stroke remains one of the leading causes of death and disability worldwide. In order to achieve the best outcome possible for the individual patient, therapies have to be administered rapidly. Here, we aim to investigate (1) the effect of collateral flow on treatment success, (2) the dynamic changes in cerebral blood flow (CBF) and the no-reflow phenomenon after stroke. We hypothesize that CBF dynamics during and after stroke may predict stroke outcome and treatment success.

Methods: For induction of experimental ischemia in two mouse strains with differences in the naive collateral network, thrombin was injected into the middle cerebral artery (MCA) of C57BL/6 and Balb-C mice. 30 min later, thrombolysis was initiated through intravenous injection of recombinant tissue plasminogen activator (rt-PA). CBF was monitored using laser speckle imaging during stroke and repeatedly until d7. Functional deficits were assessed by the sticky tape test and a composite neurological score. After final imaging and functional assessment on day 7, cardiac ink perfusion was used for visualization of blood vessels and triphhenyl tetrazolium chloride (TTC) staining for infarct size quantification.

Results: In mice with poor naïve collaterals (Balb-C), stroke led to a persisting CBF drop. Spontaneous reperfusion was only detected in few mice with good collaterals (C57BL/6). In both strains, rt-PA administration at 30 minutes after stroke improved reperfusion. The administration of rt-PA reduced infarct volume and sensorimotor deficits. Interestingly, recanalization success within the first 90 minutes was not always accompanied by tissue reperfusion and better outcome on day 7, suggesting no-reflow despite recanalization. Reperfusion of the MCA-territory was largely facilitated through ACA collaterals, which grew in diameter and number throughout the 7-day-observation period. We are currently analyzing the whole set of CBF recordings throughout day 7 to further delineate changes in perfusion and their impact on neurological outcome in the two strains.

Conclusion: We here demonstrate changes in cerebral blood flow in an experimental ischemia model of clot formation and thrombolysis, closely mimicking the situation in stroke patients. Based on our preliminary data, CBF dynamics differ in mice with a good and a poor collateral network. No-reflow despite successful recanalization of the occluded artery is a frequent phenomenon likely impacting stroke outcome. The CBF signal within the collateral territories of stroke may be a novel predictor of stroke outcome. Further analyses are under way.