

19th Annual Meeting, COSBID 2017

March 29th-31st



Kaiserin-Friedrich-Haus, Robert-Koch-Platz 7, 10115 Berlin, Germany

A special thanks to **Angelika Borkowski** for precious administrative help throughout the organization of **iCSD2017**

Welcome to Berlin for the International Conference on Spreading Depolarizations (iCSD)!

Since 2003, clinical and basic scientists from around the globe have met on a yearly basis to exchange their ideas about what we now call spreading depolarization. This started as one clinical collaboration with roots in London and Copenhagen. Soon, it blossomed into the many collaborations of the Co-Operative Studies on Brain Injury Depolarizations (COSBID) and cultivated what is increasingly recognized as a paradigmatic shift in the clinical and basic research of cerebral injuries. One noteworthy manifestation of these developments is the first special issue dedicated to spreading depolarizations in one of the leading journals of the field, the Journal of Cerebral Blood Flow and Metabolism. This will appear in April.

Last July in Albuquerque, New Mexico, those that have gathered under the umbrella of COSBID over the years decided to give the meetings an official name that attracts new attendees and their novel contributions, and also recognizes spreading depolarization as a broad field of study in its own right. It was thus decided to call this 19th meeting the “International Conference on Spreading Depolarizations” (iCSD). The particular idea was to build and strengthen bridges with the many overlapping fields of clinical and basic science inquiry. This includes research on ischaemic and haemorrhagic stroke, traumatic brain injury, migraine, epilepsy and cardiac arrest, various medical disciplines such as neurosurgery, neurology, anaesthesiology, radiology, internal medicine, epidemiology, physiology and pharmacology, and technology development in electrophysiology, monitoring devices and sensors, and neuroimaging.

We hope that we have achieved these goals in assembling a diverse and exciting program. As always, we are especially grateful to our industry partners whose contributions are vital not only for organizing the conference, but also for advancing technologies and discovery.

We hope you have an enjoyable and stimulating time with us!

The Scientific Organizing Committee

Jens Dreier

Johannes Woitzik

Oliver Sakowitz

Martin Fabricius

Martin Lauritzen

Schedule:

Wednesday, 29.03.2017

13:30 - 14:00	Registration
14:00 - 14:10	Jens P. Dreier – Welcome
14:10 - 14:30	Matthias Endres – Opening lecture
14:10 - 16:10	Session 1: Translation - Subarachnoid Hemorrhage
16:10 - 16:40	Coffee break
16:40 - 17:20	Keynote lecture 1
17:30 - 21:00	Welcome

Thursday, 30.03.2017

09:00 - 11:00	Session 2: Translation - Glutamate
11:00 - 11:30	Coffee break
11:30 - 12:10	Keynote lecture 2
12:10 - 13:30	Session 3: Translation - Ischemic Stroke
13:30 - 15:00	Lunch
15:00 - 15:40	Keynote lecture 3
15:40 - 16:40	Session 4: Translation - Migraine
16:40 - 17:10	Coffee break
17:10 - 18:30	Session 5: Spreading Depolarization Continuum
20:00 - 00:00	Dinner

Friday, 31.03.2017

09:00 - 11:00	Session 6: Overlaps between Spreading Depolarization and Epilepsy
11:00 - 11:30	Coffee break
11:30 - 12:10	Keynote lecture 4
12:10 - 13:10	Session 7: Poster and Industry Session
13:10 - 14:30	Lunch
14:30 - 15:10	Keynote lecture 5
15:10 - 16:10	Session 8a: Imaging and Electrophysiology
16:10 - 16:40	Coffee break
16:40 - 17:20	Session 8b: Imaging and Electrophysiology
17:20 - 18:25	Study reports and the next big challenges
18:25 - 18:35	Uwe Heinemann prize for young investigators
18:35 - 19:00	Announcement of 2018 meeting, Close of 2017 meeting

Detailed Schedule:

Wednesday, 29.03.2017

13:30 - 14:00 **Registration**

14:00 - 14:10 **Jens P. Dreier** - Welcome

Opening lecture

14:10 - 14:30 **Matthias Endres** - Department of Neurology,
Charité –Universitätsmedizin Berlin, Germany
Translational stroke research at the Center for Stroke Research Berlin

Session 1: Translation - Subarachnoid Hemorrhage

Chairs: Andrew Carlson, Oliver Sakowitz

14:30 - 14:50 **Jed A. Hartings** - University of Cincinnati, OH, USA
Subarachnoid blood acutely induces spreading depolarizations and early cortical infarction in the human and swine brain

14:50 - 15:10 **Sebastian Major** - Charité - Universitätsmedizin Berlin, Germany
A case of complicated migraine auras in temporal association with electrocorticographically recorded spreading depolarizations

15:10 - 15:30 **Tristan Stani** - Oregon Health and Science University, Portland, OR, USA
Correlation of glymphatic flow and cerebral blood flow following subarachnoid hemorrhage

15:30 - 15:50 **Kazutaka Sugimoto** - Yamaguchi University, Yamaguchi, Japan
Effect of cilostazol on delayed cerebral ischemia and spreading depolarization

15:50 - 16:10 **Raimund Helbok** - Medical University Innsbruck, Austria
Update on the Newton Trial

16:10 - 16:40 **Coffee break**

Keynote lecture 1

16:40 - 17:20 **Brian MacVicar** - Department of Psychiatry, University of British Columbia, Vancouver, Canada
New mechanisms underlying neuronal edema and glutamate release during spreading depolarization

17:30 - 21:00 **Welcome** - Kaiserin-Friedrich-Stiftung

Thursday, 30.03.2017

Session 2: Translation - Glutamate

Chairs: Sergei Kirov, Bill Shuttleworth

- 09:00 - 09:20 **Stéphane Marinesco** - Université Claude Bernard, Lyon, France
Minimally invasive microelectrode biosensors reveal different neurochemical signature of spreading depolarization in rat cortex
- 09:20 - 09:40 **Baptiste Balança** - Université Claude Bernard, Lyon, France
Glutamate and D-serine dynamics during trauma-induced cortical spreading depolarizations
- 09:40 - 10:00 **Edgar Santos** - University of Heidelberg, Germany
Effects of S-ketamine in the incidence, hemodynamic and electrical characteristics of spreading depolarization in gyrencephalic swine models
- 10:00 - 10:20 **Renán Sánchez-Porras** - University of Heidelberg, Germany
Intrinsic Optical Signal Imaging reveals a variety of Hemodynamic Responses to Spreading Depolarizations in the Gyrencephalic Swine Brain
- 10:20 - 10:40 **Johannes Woitzik** - Charité - Universitätsmedizin Berlin, Germany
Occurrence of Spreading Depolarization and impact on delayed infarct progression after malignant hemispheric stroke in C57/bl6 mice
- 10:40 - 11:00 **Andrew P. Carlson** - University of New Mexico, Albuquerque, NM, USA
Dose Threshold for Ketamine Suppression of CSD: Results of the Spreading Depolarization and Ketamine Suppression (SAKS) Trial.
- 11:00 - 11:30 **Coffee break**

Keynote lecture 2

- 11:30 - 12:10 **Tobias Kurth** - Institute of Public Health, Charité -
Universitätsmedizin Berlin, Germany
Consequence of migraine aura on vascular events on the population level

Session 3: Translation - Ischemic Stroke

Chairs: Cenk Ayata, Rudolf Graf

- 12:10 - 12:30 **Fumiaki Oka** - Harvard Medical School, Charlestown, MA, USA
CADASIL Mutations Increase Stroke Vulnerability
- 12:30 - 12:50 **Karl Schoknecht** - Charité - Universitätsmedizin Berlin, Germany
Electrophysiological evidence of a peri-ischemic transition zone in the rat photothrombosis model
- 12:50 - 13:10 **Ákos Menyhárt** - University of Szeged, Hungary
Spontaneous spreading depolarization augments tissue acidosis in the young and aged ischemic rat cerebral cortex
- 13:10 - 13:30 **Alan Urban** - University of Leuven, Belgium
Mapping of cortico-thalamic circuits at high spatiotemporal resolution during a spreading depolarization with functional ultrasound imaging and flexible electrode array in rats
- 13:30 - 15:00 **Lunch**

Keynote lecture 3

- 15:00 - 15:40 **Arn van den Maagdenberg** - Department of Human Genetics, Leiden University Medical Center, The Netherlands
Cortical spreading depression: investigations in transgenic mice

Session 4: Translation - Migraine

Chairs: Martin Lauritzen, Anthony Strong

- 15:40 - 16:00 **Clemens Reiffurth** - Charité - Universitätsmedizin Berlin, Germany
Deficiency of Na,K-ATPase alpha isoforms differentially modulates the threshold for spreading depolarizations in mice
- 16:00 - 16:20 **KC Brennan** - University of Utah, Salt Lake City, UT, USA
Integrating spreading depolarizations in the circuit dysfunction of migraine
- 16:20 - 16:40 **Lars Neeb** - Charité - Universitätsmedizin Berlin, Germany
Functional and structural brain alterations in chronic migraine – implications for a progressive disease?
- 16:40 - 17:10 **Coffee break**

Session 5: Spreading Depolarization Continuum

Chairs: Oscar Herreras, Frank Richter

- 17:10 - 17:30 **Omer Revah** - The Hebrew University of Jerusalem, Rehovot, Israel
The earliest neuronal responses to hypoxia in the neocortical circuit are glutamate-dependent
- 17:30 - 17:50 **Michael Gutnick** - The Hebrew University of Jerusalem, Rehovot, Israel
Functional impairment of coding capabilities of neocortical neurons that have survived anoxic depolarization
- 17:50 - 18:10 **Sergei A. Kirov** - Medical College of Georgia, Augusta, Georgia, USA
Spreading depolarization-induced disruption of dendritic ultrastructure in the murine neocortex revealed by quantitative serial section electron microscopy.
- 18:10 - 18:30 **Krzysztof Kucharz** - University of Copenhagen, Denmark
Recurrent fission-fusion of neuronal endoplasmic reticulum by synaptic excitation in physiology and in CSD
- 20:00** **Dinner – Hörsaalruine**
(Meeting at the conference venue at 19:45 or, please, go directly to the Hörsaalruine using the map on page 72)

Friday, 31.03.2017

Session 6: Overlaps between Spreading Depolarization and Epilepsy

Chairs: Martin Fabricius, Raimund Helbok

- 09:00 - 09:20 **Daniel Torres** - Cajal Institute - CSIC, Madrid, Spain
Irradiation of abnormal activity across multiple networks from discrete foci undergoing spreading depolarization or epileptic activity in rodents
- 09:20 - 09:40 **Kristina Lippmann** - Leipzig University, Leipzig, Germany
Epileptiform activity in the blood-brain barrier disrupted hippocampus is associated with altered network oscillations and synaptic plasticity
- 09:40 - 10:00 **Eszter Farkas** - University of Szeged, Hungary
Ictal activity associated with spreading depolarization in the aged rat brain: reality or illusion?
- 10:00 - 10:20 **Richard Kovacs** - Charité - Universitätsmedizin Berlin, Germany
Seizure-induced pericytic injury is associated with neurovascular decoupling and opening of the blood-brain barrier
- 10:20 - 10:40 **Lila Khennouf** - University of Copenhagen, Denmark
Role of capillary pericytes and penetrating arteriole during cortical spreading depression
- 10:40 - 11:00 **Britta E. Lindquist** - University of New Mexico, Albuquerque, NM, USA
Adenosine A_{2A} receptor activation contributes to vasodilation of cerebral parenchymal arterioles following spreading depolarization
- 11:00 - 11:30 **Coffee break**

Keynote lecture 4

- 11:30 - 12:10 **Alon Friedman** - Department of Medical Neuroscience, Dalhousie University, Halifax, Canada
Blood-brain barrier dysfunction in the injured brain: from animal experiments to the COSBID study

Session 7: Poster and Industry Session

- 12:10 - 13:10 **Sharon L. Jewell** - King's College London, UK
Tracking membrane potential changes during spreading depolarisations: A case report
- 12:10 - 13:10 **Nielsen Lagumersindez Denis** - Charité - Universitätsmedizin Berlin, Germany
Effect of reperfusion on the occurrence of spreading depolarizations in a model of focal transient cerebral ischemia in rats
- 12:10 - 13:10 **Katelyn M. Reinhart** - University of New Mexico, Albuquerque, NM, USA
Influence of tissue metabolic status on Ca^{2+} and glutamate accumulation during spreading depolarization
- 12:10 - 13:10 **Dániel Péter Varga** - University of Szeged, Hungary
Contribution of prostanoid signaling to the evolution of spreading depolarization and the associated cerebral blood flow response
- 13:10 - 14:30 **Lunch**

Keynote lecture 5

- 14:30 - 15:10 **Arno Villringer** - Department of Neurology, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany
Beyond "activation" and "deactivation": How to assess blood flow, oxygen metabolism, and neurophysiology noninvasively with MRI

Session 8: Imaging and Electrophysiology

Chairs: KC Brennan, Eszter Farkas

- 15:10 - 15:30 **Daniel Kondziella** - Rigshospitalet, Copenhagen University Hospital, Denmark
Functional MRI for Assessment of the Default Mode Network in Acute Brain Injury
- 15:30 - 15:50 **Ramani Balu** - University of Pennsylvania, Philadelphia, PA, USA
Putative spreading depolarizations after diffuse hypoxic-ischemic brain injury identified with invasive continuous cerebral perfusion monitoring

- 15:50 - 16:10 **Frank Richter** - Friedrich Schiller University Jena, Germany
Substance P is able to elicit cortical spreading depolarization (CSD) in adult rats – a mechanism responsible for aggravation of cortical damage after brain injury or stroke?
- 16:10 - 16:40 **Coffee break**
- 16:40 - 17:00 **Sharon L. Jewell** - King's College London, UK
Tracking membrane potential changes in the in-vivo human brain Part I: Monitoring changes in baseline membrane potential and during the recovery cycle of excitability.
- 17:00 - 17:20 **Jordi Serra** - King's College London, UK
Tracking membrane potential changes in the in-vivo human brain Part II: Monitoring changes in post-activation afterpotentials.
- 17:20 – 18:25 Study reports and the next big challenges**
Chair: Jed A. Hartings
- 17:20 - 17:35 **Renán Sánchez-Porras, Oliver W. Sakowitz**
CENTER-TBI
- 17:35 - 17:40 **Jens P. Dreier**
DISCHARGE-1
- 17:40 - 17:48 **Johannes Woitzik**
MHS
- 18:25 - 18:35 Uwe Heineman Young Scientist Prize**
- 18:25 - 18:30 **Michael Gutnick** – Uwe Heineman memorial speech
- 18:35 - 19:00 **Announcement of 2018 meeting, Close of 2017 meeting**

Abstracts

Subarachnoid blood acutely induces spreading depolarizations and early cortical infarction in the human and swine brain

Jed A. Hartings^{1,2*}, Jonathan York¹, Jason M. Hinzman¹, Bryan Krueger¹, Maren Winkler³, Sebastian Major^{3,4,5}, Viktor Horst³, Paul Jahnke⁶, Johannes Woitzik⁷, Eric Mahoney¹, Yifeng Du⁸, Matthew Hagen⁹, Jianxiang Jiang⁸, Jens P. Dreier^{3,4,5}

¹Department of Neurosurgery and ²Department of Pathology and Laboratory Medicine, University of Cincinnati (UC) College of Medicine, Cincinnati, OH; ³UC Gardner Neuroscience Institute and Mayfield Clinic, Cincinnati, OH; ⁴Division of Pharmaceutical Sciences, UC College of Pharmacy, Cincinnati, OH; ⁵Center for Stroke Research Berlin, ⁶Department of Neurology, ⁷Department of Experimental Neurology, ⁸Department of Radiology and ⁹Department of Neurosurgery, Charité University Medicine Berlin, Germany

Early cortical infarction is common in poor-grade patients after aneurysmal subarachnoid hemorrhage, yet there are no animal models of these lesions and mechanisms are unknown. Here we investigated acute sequelae of subarachnoid hemorrhage in the gyrencephalic brain of anesthetized juvenile swine by multi-modal neuromonitoring and post-mortem studies. Subarachnoid infusion of 1-2 ml of fresh arterial blood over a cortical sulcus caused spreading depolarizations (SDs) in 13/17 animals and temporal clusters of SDs in 7 animals in a 6-hr monitoring period. SD clusters were significantly associated with thicker sulcal clots ($P < 0.05$) and a high likelihood of cortical infarcts (5/7 compared to 2/10 in swine without clusters, $P < 0.06$). In a second cohort, sulcal infusion of clotted blood produced thicker (median: 6.2 mm) clots, extensive infarction of circumscribed cortex, and SDs in 5/6 animals. The association of SDs with early brain injury was then investigated in 23 patients who underwent early magnetic resonance imaging and frontal electrocorticography after repair of ruptured anterior communicating artery aneurysms. Patients with any frontal lobe brain lesion and those with frontal infarcts only were both significantly more likely to have SDs [10/12 (83%) and 6/7 (86%), respectively] than those without brain lesions (1/11, 9%) (P 's < 0.001 and < 0.05 , respectively). These results demonstrate an association between SDs and early cortical infarction after aneurysmal subarachnoid hemorrhage and establish a clinically relevant model to investigate causal sequences and potential therapeutic interventions.

A case of complicated migraine auras in temporal association with electrocorticographically recorded spreading depolarizations

Sebastian Major^{1,2,3}, Denny Milakara¹, Johannes Woitzik⁴, Jens P. Dreier^{1,2,3}

¹Center for Stroke Research Berlin, ²Department of Experimental Neurology, ³Department of Neurology, ⁴Department of Neurosurgery, Charité - Universitätsmedizin Berlin, Berlin, Germany

In the last 15 years more than 500 patients with stroke and brain trauma underwent neuromonitoring of spreading depolarizations (SD) in neurocritical care within the framework of the Co-Operative Studies on Brain Injury Depolarizations (COSBID). Unequivocal electrophysiological evidence was found that SDs are involved in the pathophysiology of these human diseases in accordance with previous animal research. Preliminary clinical evidence in patients with subarachnoid hemorrhage (SAH) suggested moreover that clusters of SDs can be associated with waxing and waning and also permanent severe focal and global neurological deficits. However, Leão and Morison also proposed in 1945 that SD is the pathophysiological correlate of the migraine aura. For obvious reasons, it is currently not possible to implant the invasive recording tools for the measurement of SDs in patients before the occurrence of a migraine aura. Therefore, the electrophysiological proof whether or not SD is its pathophysiological correlate is still lacking. Yet, it is somewhat puzzling that not a single case report has described the patient percept of a migraine aura when SD was recorded in awake patients with cerebral injury. Here we present a 56-year old female who had suffered from SAH due to rupture of a left middle cerebral artery aneurysm. A subdural electrode array was implanted over the left frontal lobe after surgical clip ligation of the aneurysm. Eleven days after the initial hemorrhage the fully awake and oriented patient developed dysarthria followed first by facial paresis and then paresis of her right arm some minutes later in presence of a neurologist. Several minutes after symptom onset, SD was seen on the monitor propagating in caudal-rostral direction. Similar events recurred twice with full recovery in between and thereafter. To our knowledge, this is the first case report of complicated migraine auras in temporal association with electrocorticographically recorded SDs.

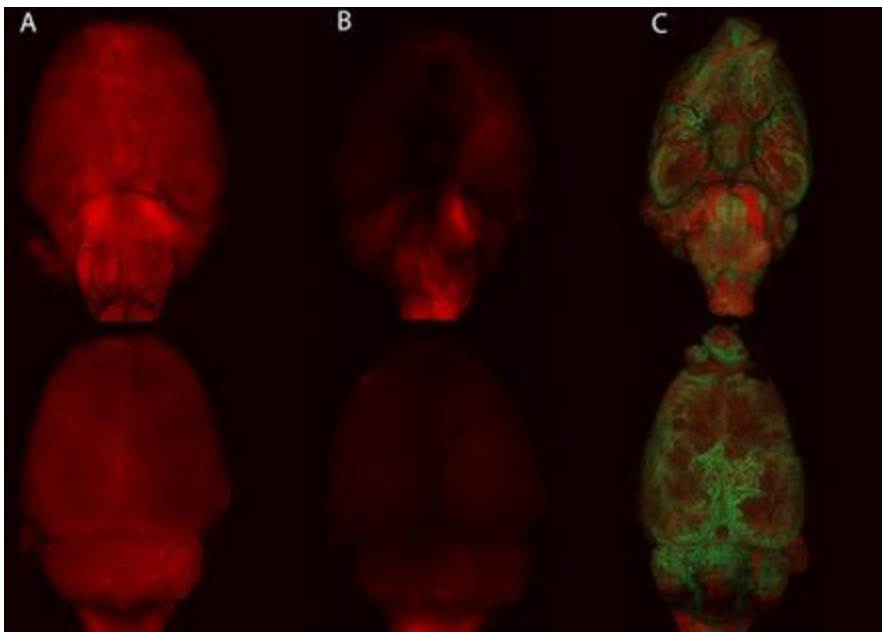
Correlation of glymphatic flow and cerebral blood flow following subarachnoid hemorrhage

Tristan Stani, MD¹, Jeff Iliff, PhD², Justin Cetas MD, PhD¹

¹Department of Neurological Surgery, ²Department of Anesthesia and Perioperative Medicine, Oregon Health and Science University Portland, OR, USA

Introduction: Subarachnoid hemorrhage (SAH) produces profound disruptions in cerebral blood flow (CBF), with spreading depolarization as a possible mechanism causing impaired neurovascular coupling (Koide et al: Subarachnoid hemorrhage, spreading depolarizations and impaired neurovascular coupling. **Stroke Res Treat** **2013**: 819340). Recent data also shows that brain-wide glymphatic CSF flux is disrupted after SAH (Gaberel et al: Impaired Glymphatic Perfusion After Strokes Revealed by Contrast-Enhanced MRI: a new target for fibrinolysis? **Stroke** **45**:3092-6, 2014). This finding present the possibility for new therapeutic interventions but further characterization of the potential relationships between glymphatic function and CBF is needed.

Methods: To model SAH, autologous blood was injected into the prechiasmatic cistern of adult male rats (Figure 1, B). A second treatment group received injection of inert fluorescent beads approximately the size of blood cells (Figure 1, C). Controls received artificial CSF injection (Figure 1, A).



Blood flow changes following injection were assessed using laser speckle imaging through a thin skull preparation. 20 μ l of 1% Evans Blue (EB) was injected into the cisterna magna 30 minutes after prechiasmatic cistern injections. Rat brains were perfused and fixed 60 minutes after injections. The brain surfaces were imaged under a fluorescent microscope (Figure 1). EB fluorescence intensity was calculated over multiple brain regions and correlated to CBF changes.

Results: Injection of subarachnoid blood caused various changes in CBF. Surface flow of EB CSF tracer was correlated to CBF changes. Total EB signal was decreased across the entire brain surface in all SAH-model animals compared to controls. Injection of beads caused similar disruption in EB tracer flow. Regional sub-analyses between EB signal and CBF were assessed.

Conclusion: SAH as well as subarachnoid bead injection both led to decreased perivascular CSF flow. Glymphatic disruption may relate to alterations in CBF. Glymphatic flow restoration may present a new therapeutic target in SAH.

Effect of cilostazol on delayed cerebral ischemia and spreading depolarization

Kazutaka Sugimoto, Satoshi Shirao, Takao Inoue, Eiichi Suehiro, Fumiaki Oka, Akiko Kawano, Hideyuki Ishihara, Sadahiro Nomura, Michiyasu Suzuki

Department of Neurosurgery, Yamaguchi University graduate school of medicine, Yamaguchi, Japan

Object: Cilostazol, a phosphodiesterase 3 inhibitor, has recently been reported to have a prophylactic effect on symptomatic vasospasm after aneurysmal SAH (aSAH). In addition, the association between Spreading depolarization (SD) and delayed cerebral ischemia (DCI) is reported and there is a possibility of a new therapeutic target.

In this study, we investigated (1) the effect of cilostazol on DCI after aSAH, and (2) on SD

Methods: The subjects were 34 patients with aSAH who were treated by clipping within 72 hours from onset. Patients were randomly classified into a group given 100 mg cilostazol twice daily (cilostazol group, n=17) and a control group that did not receive cilostazol (n=17). In all patients, SD recording was conducted using continuous recording of the electrocorticogram (ECoG) with 6-electrode (linear array) subdural strips. The frequencies of DCI and SD were compared between the two groups. We also evaluated the duration of Spreading depression. DCI was defined as presentation with a delayed ischemic neurological deficit or delayed infarction.

Results: DCI occurred in 3 patient (18%) in the cilostazol group and in 7 patients (41%) in the control group. SD occurred in 11 patient (65%) in the cilostazol group and in 12 patients (71%) in the control group. The total incidences of SD from day 4 to day 14 were 132 in the cilostazol group and 439 in the control group. The total depression duration of all recording day were 761.1 minutes in the cilostazol group and 4327.8 minutes in the control group. These findings indicate that cilostazol may ameliorate DCI and suppresses the occurrence of SD.

Conclusions: Cilostazol is effective for prevention of DCI after aSAH and attenuation of the incidence of SD. Cilostazol may suppress SD-induced microcirculatory disturbance and may reduce DCI.

Minimally invasive microelectrode biosensors reveal different neurochemical signature of spreading depolarization in rat cortex.

Anne MEILLER^{1,2}, Charles CHATARD^{1,3}, Baptiste Balança¹, Andrei SABAC³, Stéphane MARINESCO^{1,2}

¹Lyon neuroscience research center, team TIGER, Université Claude Bernard, Lyon, France; ²Lyon neuroscience research center, AniRA-Neurochem platform, Université Claude Bernard, ³INSA Lyon – INL, Campus LyonTech-La Doua, Villeurbanne, France.

Monitoring the chemical composition of the brain interstitial fluid is an important challenge for both pre-clinical and clinical research on brain injury. Microelectrode biosensors are a promising technique with a temporal resolution in the order of seconds. Here, ultra-microelectrodes based on platinized carbon fibers were fabricated to obtain biosensors with less than 15 µm external diameter. Platinization was achieved by sputtering a 10 nm Cr adhesion layer followed by 100 nm of platinum. Platinized carbon fibers were then encased in a glass micropipette and covered with electropolymerized poly-phenylenediamine for selectivity, and covalently immobilized oxidase enzymes (glucose oxidase, lactate oxidase, D-amino acid oxidase or glutamate oxidase). After implantation in the rat parietal cortex, such biosensors detected a smaller basal lactate concentration and a slower diffusion of glucose and D-serine through the blood brain barrier compared to more conventional biosensors with 100 µm external diameter. Interestingly, spreading depolarization (SD) produced a smaller increase in lactate, a larger decrease in glucose, and a larger increase in D-serine at platinized carbon fibers microelectrode biosensors compared to larger sensors. Therefore, the neurochemical signature of SDs was significantly different when estimated with these new minimally invasive biosensors. Such small devices avoid major mechanical injury to blood vessels, preserve the blood brain barrier at the site of implantation, and therefore, provide more accurate measurements from the brain interstitial fluid. Developing smaller, less invasive probes for brain monitoring is therefore an important challenge in order to obtain meaningful information about the cellular mechanisms at work during brain injury.

Glutamate and D-serine dynamics during trauma-induced cortical spreading depolarizations

Baptiste BALANCA¹, Thomas LIEUTAUD¹, Clélia ALLIOUX¹, Anne MEILLER^{1,2}, Stéphane MARINESCO^{1,2}

¹Lyon neuroscience research center, team TIGER, Université Claude Bernard, Lyon, France; ²Lyon neuroscience research center, AniRA-Neurochem platform, Université Claude Bernard

Cortical spreading depolarizations (SDs) are waves of massive depolarization travelling through the cortex at a speed of 2-3 mm/min. They are observed after severe brain injury in humans and animals and associated with poor outcome. N-methyl-D-aspartate receptors (NMDAR) are involved in SD induction and/or propagation, and excitotoxic NMDAR overactivation could play an important role in neuronal injury following severe traumatic brain injury (TBI). However monitoring glutamate extracellular concentration is a technical challenge and the range of glutamate elevation during SDs remains a matter of debate. In addition to glutamate, D-serine is another endogenous NMDAR agonist and its concentrations during SDs and after TBI remain unknown. Here, we monitored glutamate and D-serine dynamics in the cortex of male Sprague-Dawley rats during 5h following a severe (3.8 atmosphere) brain lateral fluid percussion injury. We used enzyme microelectrode biosensors with an external diameter of 40 μm that are known to preserve blood-brain barrier integrity. In addition, cortical perfusion was assessed by laser Doppler flowmetry (LDF) and local field potentials were monitored in direct current (DC) mode to detect SDs. SDs occurred spontaneously after severe TBI at a rate of about 2 SDs in the first 5h following TBI, and were associated with a hyperemic response. The basal extracellular glutamate levels displayed a more than 10 fold increase ($10.4 \pm 2.3 \mu\text{M}$ after TBI vs submicromolar levels in control animals), whereas D-Serine levels, by contrast, were severely diminished (not detectable after TBI vs 2-3 μM in control animals). Glutamate concentration increased dramatically during SDs ($76.3 \pm 25.4 \mu\text{M}$) whereas D-serine was little affected. These data indicate that NMDARs after severe TBI are under the opposite influence of increased glutamate (both tonic elevation of basal glutamate and glutamate surges during SDs) and decreased D-serine levels. The overall effects of these changes in amino acid concentrations on NMDAR activation and excitotoxicity remain to be elucidated, but it is possible that the decrease in D-serine that we observed provides a compensatory protective mechanism against elevated glutamate.

Effects of S-ketamine in the incidence, hemodynamic and electrical characteristics of spreading depolarization in gyrencephalic swine models

Edgar Santos¹, Arturo Olivares Rivera¹, Renan Sánchez-Porras¹, Modar Kentar¹, Martina Mann¹, Oliver W. Sakowitz^{1,2} Andreas W. Unterberg¹

¹Department of Neurosurgery, University of Heidelberg, Heidelberg, ²Klinikum Ludwigsburg, Ludwigsburg, Germany

Electrical and hemodynamic characteristics of spreading depolarizations (SDs) can be affected by the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine, which has been shown to produce neuroprotection in animals with lissencephalic brains. In a previous analysis of Subarachnoid Hemorrhage Patients from Heidelberg and Berlin, a mean of 2.7mg/kg/h s-ketamine reduced the SD incidence from 1.87 to 0.68 SDs/day (reduction to 36.6%). The effectiveness of SD-targeted neuroprotective therapies could be evaluated in real time by measuring SD incidence. Using gyrencephalic brain models we might translate the information about effectivity of ketamine infusion to reduce the SD incidence more easily into the patients.

We investigated the effect of s-ketamine on SD incidence and characteristics in two translational porcine models. First a KCl model and in an middle cerebral artery occlusion model (MCAo).

In both animal models, SDs were monitored using electrocorticography (ECoG) and large field-of-view movement-compensated intrinsic optical signal (IOS) imaging, which enabled us to perform a long-term analysis of blood volume signals in regions of interest. In the first animal model (n=15), SDs were induced with drops of 1M KCl in both hemispheres at 1h-intervals during 18h (Group 1: control, Group 2: ketamine 2mg/kg/h, Group 3: 4mg/kg/h, each n=5). In a second model, the left middle cerebral artery was occluded and SDs occurred spontaneously. Animals were randomly assigned to either receive 5mg/kg/h ketamine or not (Groups 4 and 5, each n=5) and were monitored over 30h.

Ketamine reduced the incidence of SDs in both porcine models (reduction to 35.7% in the KCl and 68.7% in the MCAo model using ketamine 4mg/kg/h). We also found significant changes in the electrical and hemodynamic characteristics (amplitude, duration, expansion, etc.). In the KCl model, doses above the recommended therapeutic range (>2mg/kg/h) were more effective. Following MCAo, ketamine was less effective in reducing the SD incidence. Ketamine has the capability to reduce the incidence and characteristics of SDs in gyrencephalic brain of both porcine model and SAH patients. Most effective doses are above the recommended therapeutic range for sedation. More information is required before proceeding to a clinical trial.

Intrinsic Optical Signal Imaging reveals a variety of Hemodynamic Responses to Spreading Depolarizations in the Gyrencephalic Swine Brain

Renán Sánchez-Porras¹, Edgar Santos¹, Martina Mann¹, Arturo Olivares-Rivera¹, Alejandro Alvarez-Ayala¹, Modar Kentar¹, Roland Zerelles¹, Oliver W. Sakowitz^{1,2} Andreas W. Unterberg¹

¹*Department of Neurosurgery. University of Heidelberg, Heidelberg, Germany;* ²*Department of Neurosurgery. Klinikum Ludwigsburg, Ludwigsburg, Germany*

Four vasomotor components shaping the hemodynamic response to spreading depolarizations (SDs) have been previously described mostly in lissencephalic brains. Some exerting a dilative influence while others a constrictive. However, up to now a systematic characterization of the variety of hemodynamic responses to SD in large gyrencephalic brain is scarce.

The aim of this work was first to describe the hemodynamic patterns to SDs according to their components; and second to investigate the influence of ketamine, a N-methyl-D-aspartate (NMDA) receptor blocker, on the vasomotor components of the hemodynamic response to SD in the swine brain. Intrinsic optical signal (IOS) imaging, as a measure of cerebral blood volume changes was used to monitor the hemodynamic changes to SDs in two different models of 20 swine in total, divided into 4 groups. Additionally we validated the presence of SDs using electrocorticography. In the first model, SDs were elicited by topical application of KCl; and in the second model, they occurred spontaneously after middle cerebral artery (MCA) occlusion. Intervention with ketamine at a high dose of 4 mg/kg/h and 5 mg/kg/h was given in two groups.

After KCl stimulation SDs initiated as either concentric-radial or irregular-broken-radial waves that propagated in several semi-planar fronts. After clipping a drastic intensity change in IOS was seen in the cortex area supplied by the MCA. In this model SDs initiated mainly as irregular-broken-radial waves with semi-planar fronts. Using IOS imaging, we identify the four vasomotor components, two hyperemic and two oligemic, of the hemodynamic response to SD. The presence or absence, and the spatio-temporal order of this components resulted in the development of 13 different patterns, ranging from a monophasic pattern of either pure hyperemia or oligemia, to the development of more complex patterns consisting of 4 or 5 phases. The pharmacological intervention with ketamine influenced the magnitude and timing of the different vasomotor elements, capable to shape the hemodynamic response to SD.

The identification and characterization of the diversity of hemodynamic responses to SDs and their vasomotor components, in particular of the harmful ones, is of particular interest for the identification and/or development of therapeutic options that could contract their noxious effects.

Keywords: electrocorticography; hemodynamic response; intrinsic optical signal imaging; middle cerebral artery occlusion; spreading depolarization

Occurrence of Spreading Depolarization and impact on delayed infarct progression after malignant hemispheric stroke in C57/bl6 mice

A. Zdunczyk¹, L. Schumm¹, X. Bai¹, S. Major², P. Vajkoczy¹, J. Woitzik¹

¹ Department of Neurosurgery, Charité Universitätsmedizin Berlin; ² Department of Neurology, Charité Universitätsmedizin Berlin

Background. Spreading depolarization (SD) occurs in high frequency in patients with malignant hemispheric stroke (MHS) and is coupled either to hyperaemic or hypoaemic blood flow responses. After experimental focal ischaemia SD is a significant cause for secondary stroke progression during the initial period of infarct maturation. Due to the need of surgery SD is typically monitored 48 to 120 hours after stroke onset under clinical conditions. Currently it is not known by what extent SD contributes to stroke progression during this delayed period. In this current study we analyze neurovascular coupling and occurrence of SD in a later phase of experimental cerebral ischemia.

Methods. Permanent focal ischemia was induced by distal occlusion of the left middle cerebral artery in male C57/bl6 mice. 24h after MCA occlusion, spreading depolarization was induced with potassium chloride. The neurovascular response was measured by laser speckle contrast analysis. Infarct progression was evaluated by sequential MRI. Three study groups were analyzed: control group without SD induction, SD induction with potassium chloride and SD induction and Ketamine administration (25 mg/kg body weight i.p.).

Results. 24 hours after stroke onset we observed 0.2 ± 0.2 SD/hour. The mean duration was 1.6 ± 1.0 minutes. During potassium application the frequency and duration increased to 3.3 ± 0.7 SD/hour and 3.2 ± 3.5 minutes, respectively. Ketamin treatment reduced the number and duration to 2.4 ± 0.8 SD/hour and 1.36 ± 0.95 minutes, respectively. Neurovascular coupling was dependent on the distance from the ischemic region but did not differ between individual groups. Induction of SD significantly increased stroke volume even 24 hours after stroke onset, which could be prevented by additional ketamine treatment.

Conclusions. Induction of SD with potassium was significantly associated with stroke progression even 24 hours after stroke onset. Therefore, SD might be a significant contributor for delayed stroke progression. Ketamine might be a possible drug to prevent SD induced stroke progression.

Dose Threshold for Ketamine Suppression of CSD: Results of the Spreading Depolarization and Ketamine Suppression (SAKS) Trial.

Andrew P Carlson, MD, MS-CR¹; Mohammad Abbas, MD¹; Rob Alunday, MD¹; Fares Qeadan PhD²; Bill Shuttleworth, PhD³.

Departments of Neurosurgery¹, Internal Medicine², and Neuroscience³, University of New Mexico School of Medicine, Albuquerque, NM, 87122

Background: Retrospective clinical data and case studies support a therapeutic effect of ketamine in suppression of CSD. Animal and slice data strongly support efficacy both in terms of frequency of CSD as well as recovery from ECoG depression. We present the results of the first prospective clinical trial testing the role of ketamine used for clinical sedation on occurrence of CSD.

Methods: 10 subjects with severe traumatic brain injury (TBI) or aneurysmal subarachnoid hemorrhage (SAH) were recruited for this pilot trial. A standard ECoG strip was placed at the time of craniotomy and subjects were then placed on an alternating every 6 hour schedule of ketamine or other sedation agent. The order of treatment was randomized. The ketamine dose was adjusted to clinical effect and left at a subanesthetic basal dose if no sedation was required (0.1mg/kg/h.) CSD was then scored using standard criteria, blinded to ketamine dosing. Occurrence of CSD was then compared to the hourly dose of ketamine to determine the effect of ketamine on CSD occurrence.

Results: Successful ECoG recordings were obtained in all 10 subjects—8 with SAH and 2 with TBI. There was a total of 1642 hours of observations with adequate ECoG— 833 off ketamine and 809 on ketamine. Analysis revealed a strong dose dependent effect such that hours off ketamine or on doses of less than 1.15mg/kg/h were associated with an increased risk of CSD compared with hours on doses of 1.15mg/kg/h or more (OR=13.838, 95% CI= 1.99-1000). This odds ratio decreases with lower doses of 1.0 mg/kg/h, 0.85mg/kg/h, and 0.70mg/kg/h to a threshold of no effect at 0.55mg/kg/h (respective odds ratios: OR=4.924, 95% CI= 1.337-43.516; OR=3.323, 95% CI= 1.139-16.074; OR= 2.725, 95% CI= 1.068-9.898; OR=1.043, 95% CI=0.565- 2.135). When all ketamine data were pooled (i.e. on ketamine at any dose versus off ketamine), a non-significant overall trend toward fewer CSD during hours on ketamine ($\chi^2=3.86$, $p=0.42$) was observed. There was no significant effect of ketamine on the mean duration of depression after CSD ($F=2.62$, $p=0.11$).

Conclusions: Racemic ketamine successfully suppresses CSD at doses greater than 1.15mg/kg/h. Doses below 0.55mg/kg/h do not seem to have a significant effect. Future trials should target subjects with known CSD with doses greater than 1.15mg/kg/h. It is unclear if these effects differ from the previously reported effects using S-ketamine, which is not available in the US. This trial also supports the feasibility of using CSD as a surrogate for outcome for pilot testing of new interventions.

Funding: support from CTSC Pilot Grant #1UL1TR001449 and the UNM department of Neurosurgery

CADASIL Mutations Increase Stroke Vulnerability

Fumiaki Oka¹, Jeong-Hyun Lee¹, Izumi Yuzawa¹, Daniel von Bornstädt¹, Tao Qin¹, Katharina Eikermann-Haerter¹, Anne Joutel², Cenk Ayata^{1,3}

¹Departments of Radiology and ³Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129; ²INSERM, U1161 and Univ Paris Diderot, Sorbonne Paris Cité, UMRS 1161, Paris, F-75010, France

Objectives: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), linked to mutations in *NOTCH3* expressed predominantly in vascular smooth muscle cells in adult brain, is characterized by progressive leukoaraiosis, and recurrent ischemic strokes in young or middle aged adults. Mechanisms underlying vascular dysfunction and stroke are unclear. We aimed to characterize the focal cerebral ischemia phenotype in transgenic mice expressing naturally occurring CADASIL mutations, to elucidate the mechanisms rendering the CADASIL brain vulnerable to stroke.

Methods: We transiently (45min or 1h) occluded the middle cerebral artery by either an intraluminal filament proximally (fMCAO) or a microvascular clip distally (dMCAO) in two different CADASIL mutant mice (*NOTCH3* R90C or R169C). We recorded cerebral blood flow (CBF) using laser Doppler (LDF) or speckle flowmetry (LSF), in each stroke model, respectively. We also studied cortical electrophysiology during acute stroke, tissue and neurological outcomes 24 hours after reperfusion, and gross cerebrovascular anatomy, including the diameter of major cerebral arteries, patency of posterior communicating artery and number of pial artery anastomoses. Mutants were compared with their age-matched wild-type (WT) littermates. Group sizes indicated below include WT controls of each strain.

Results: Both R90C and R169C mutants developed significant larger infarcts (40% and 25% larger than WT, n=65 and 34, respectively, $p<0.05$) after filament MCAO. Surprisingly, neither LDF during filament MCAO nor LSF during distal MCAO showed worse CBF deficits (n=26 in R90C and n=20 in R169C), in either mutant. Cerebrovascular anatomy did not differ between R90C mutant and WT (n=17). Interestingly, peri-infarct depolarization frequency was increased (1.5 fold) in the mutants during filament MCAO ($p=0.07$, n=13). Presumably as a consequence of this, CBF threshold for tissue viability (figure) was significantly higher in R90C mutants

compared with wild type ($40\pm 1\%$ versus $33\pm 2\%$ of baseline CBF, respectively, $p=0.032$, $n=14$), suggesting increased sensitivity to ischemic injury.

Conclusions: Our results show that outcome of focal cerebral ischemia is worse in CADASIL mutants independent of cerebral hemodynamic factors or collateral function, suggesting higher parenchymal sensitivity to ischemia possibly linked to frequent peri-infarct spreading depolarization events. The latter may be a reflection of increased susceptibility to spreading depression as a hyperexcitability phenotype in CADASIL.

Electrophysiological evidence of a peri-ischemic transition zone in the rat photothrombosis model

Karl Schoknecht, MD/PhD^{1,2,6}, Uwe Heinemann¹, Alon Friedman, MD/PhD^{3,4}, Jens Dreier MD^{5,6}

¹Neuroscience Research Center, ²Institute for Neurophysiology, ⁵Center for Stroke Research Berlin (CSB), ⁶Department of Neurology and Experimental Neurology, Charité - University Medicine Berlin, Germany; ³Departments of Physiology & Cell Biology, Cognitive & Brain Sciences, the Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer-Sheva, Israel; ⁴Department of Medical Neuroscience, Dalhousie University, Halifax, Canada

Stroke is one of the leading causes of death and disability. In hospital, the ischemic penumbra may either recover or undergo delayed injury and cell death. The penumbra is hence potentially salvageable. Unfortunately, the mechanisms of delayed injury are still largely unknown. Here we investigated the influence of epileptic seizures and spreading depolarizations (SDs) on the progression of injury in the penumbra using the photothrombosis model (PT).

Cerebral ischemia was induced by the Rose bengal PT model in anaesthetized male Wistar rats. Blood-brain barrier (BBB) permeability, cerebral perfusion and cell damage were assessed through a cranial window following intravenous injection of fluorescein sodium salt (BBB permeability and cerebral perfusion) and propidium iodide (cell damage) until 4 hrs post PT. Intracortical field potentials and extracellular potassium concentrations were measured using ion-sensitive microelectrodes. Seizures and SDs were induced by topical perfusion of 4-aminopyridine (4-AP).

Topical 4-AP administration led to rapid stroke progression as hypoperfusion, BBB dysfunction and cellular damage spread beyond the primary ischemic core. Following PT, 4-AP induced repetitive seizures and superimposed SDs and SD clusters, while 4-AP never induced SDs in control, lesion-free animals. As an indicator of metabolic disturbance, peri-ischemic interictal extracellular potassium levels rose from 3 to 6.1 ± 2.0 mM ($p < 0.05$, $n = 13$), whereas interictal potassium levels remained normal in lesion-free animals. Interestingly, increased extracellular potassium levels correlated with increased BBB permeability ($R = 0.57$, $p = 0.042$, $n = 13$).

Together, our data suggest a metabolically impaired peri-ischemic transition zone in the PT model and propose a link between BBB dysfunction and extracellular potassium accumulation.

Spontaneous spreading depolarization augments tissue acidosis in the young and aged ischemic rat cerebral cortex

Ákos Menyhárt, Dániel Zölei-Szénási, Tamás Puskás, Péter Makra, Ferenc Bari, Eszter Farkas

Department of Medical Physics and Informatics, Faculty of Medicine, University of Szeged, Korányi fasor 9, Szeged, Hungary

Spreading depolarizations (SDs) occur spontaneously in the cerebral cortex of malignant hemispheric stroke patients. SDs exacerbate focal ischemic injury by converting zones of the viable but non-functional ischemic penumbra to the core region beyond rescue. Yet the SD-related mechanisms to mediate neurodegeneration remain poorly understood. The regulation of pH changes of neurons is crucial, because acid loading makes them susceptible for injury. Therefore we set out to evaluate intra- and extracellular pH changes associated with spontaneous SDs in the ischemic cortex of rats.

Open or closed cranial windows were mounted on the parietal bone of isoflurane-anesthetized 2-months- or 18-20-month-old Sprague-Dawley rats. SD-related variations in extracellular pH together with changes in local cerebral blood flow were acquired with pH-sensitive microelectrodes and laser-Doppler flowmetry (n=17). In additional imaging experiments, SD-coupled intracellular pH- and perfusion changes were monitored relying on the fluorescence intensity of a pH indicator dye (Neutral Red), and laser speckle contrast analysis (n=20). After a baseline period of 50 min, ischemia of a level typical of penumbra regions was achieved by bilateral common carotid artery occlusion. SDs that evolved spontaneously within minutes following ischemia induction were analyzed in details.

A single spontaneous SD event occurred within 2 min after ischemia induction in a total number of 15 out of 33 experiments. A drop of CBF below 22-23% due to ischemia induction, or ischemia-induced acidosis greater than 0.2 pH units favored SD occurrence. The SD-related acidosis was superimposed on ischemia-induced acidosis thereby transiently decreasing pHe from the ischemia-related value of 6.93 ± 0.09 to as low as $\text{pH } 6.48 \pm 0.16$ in the young group, and from the ischemia-caused $\text{pH } 7.06 \pm 0.10$ to 6.76 ± 0.20 in the old animals. Tissue pH in young animals measured over 10 min after spontaneous SD settled to a value more acidic than that produced by ischemia alone at a corresponding point of time ($\text{pH } 7.09 \pm 0.09$ vs. 7.29 ± 0.16), which was further worsened

by age, as pHe after spontaneous SD was maintained at an average of pH 6.94 ± 0.08 in the old animals.

In conclusion, spontaneous SDs increase acid accumulation under ischemic penumbra conditions to toxic levels characteristic of the ischemic core, which is suggested as a mechanisms to extend ischemic lesions. Further we show, that low tissue pH is maintained following SD events, which is known to decrease the threshold of acid-induced cell death. Finally, the aged brain may be at higher risk for SD-related injury, because the recovery of tissue pH after SD is hampered.

Mapping of cortico-thalamic circuits at high spatiotemporal resolution during a spreading depolarization with functional ultrasound imaging and flexible electrode array in rats

Blanche PIRAUX¹, Clément BRUNNER¹, Gabriel MONTALDO¹, Dries KIL², Frederik CEYSSENS², Robert PUERS² and Alan URBAN¹

¹Laboratory of neural circuits, NERF, IMEC, VIB, KU Leuven, Leuven, Belgium; ²ESAT-MICAS Department, KU Leuven, Leuven, Belgium.

Spreading depolarization (SD) is a slowly propagated wave of depolarization followed by suppression of brain activity that triggers complex events involving massive changes in both neural and vascular function. SD propagation has been extensively described in the cortex but little is still known about the spreading and effects of a SD in subcortical areas including the thalamus. For the first time, we combined functional ultrasound imaging (fUSi) with an ultrasound compatible flexible electrode array (25mm²) to map hemodynamics and electrical changes at both high spatiotemporal resolution (80µm pixel size, real time) and large scale in anesthetized rats. We observed that a SD triggered in the primary visual cortex produced a quadriphasic hemodynamic and neuronal response in the cortex. In the first phase, we observed a large increase (+50% wave A) of cerebral blood volume (CBV) concomitant with a burst of action potentials. A transient hypoperfusion was then correlated with the beginning of the neuronal silence, followed by a massive (+150% wave B) increase of CBV while neurons remain silent. A fourth, long-lasting phase of hypoperfusion was associated with a progressive recovery of electrical activity before reaching the baseline levels. In the thalamus, we observed a biphasic hemodynamic profile with 2 large increase of CBV (+100% and +70%) slightly delayed with those in the cortex and followed by a weak hypoperfusion. To further understand the cellular components involved in SD, we treated rats with either a NMDA antagonist (memantine) or with a neuroprotective glial cell modulator (propentofylline) and we observed a differential effect on hemodynamic waves A and B in the cortex but not in the thalamus. This result suggests that mechanisms of CBV increase during SD may differs between the cortex and the thalamus. Our findings demonstrate that fUSi combined with large scale electrical recording is a promising approach to better understand SD in pathologies such as migraine, stroke, subarachnoid hemorrhage and traumatic brain injury.

Deficiency of Na,K-ATPase alpha isoforms differentially modulates the threshold for spreading depolarizations in mice

C. Reiffurth^{1,2}, M. Alam⁵, M. Zahedi-Khorasani⁴, S. Major^{1,2,3}, and J.P. Dreier^{1,2,3}

¹Department of Experimental Neurology, ²Center for Stroke Research, ³Department of Neurology, Charité University Medicine Berlin, Germany; ⁴Laboratory of Cerebrovascular Research, Physiological Research Center, University of Medical Sciences, Semnan, Iran; ⁵Department of Neurosurgery, Hannover Medical School, Hannover, Germany

Objectives: Mutations in ATP1A2, the gene encoding the Na,K-ATPase alpha2 subunit, have been identified in patients suffering from a severe form of migraine with aura: familial hemiplegic migraine type 2 (FHM2). It has been hypothesized that spreading depolarization (SD, spreading depression), the neurophysiological process underlying migraine aura, might be facilitated by a loss of function of a single allele of the gene encoding the alpha2 subunit. To address the question whether a specific reduction of the alpha2 isoform affects the threshold for SD ignition we have employed heterozygous knockout mice lacking one copy of the $\alpha 2$ subunit encoding allele (alpha2+/-) and provoked SD by various stimuli.

Methods: In acute brain slices, SD was triggered focally by droplet application of 1 M KCl solution, by electrical stimulation or by stepwise increasing the K⁺ concentration in the bathing solution. We recorded changes in extracellular K⁺ concentration, the accompanying slow extracellular potential shift, as well as changes in intrinsic optical signals to assess spatiotemporal patterns. To further investigate whether the observed effects were specific for a reduced amount of the alpha2 isoform, alpha1 and alpha3 heterozygous (alpha1+/- and alpha3+/-) mice were included in this study.

Results: We found a slightly but significantly lowered ($P < 0.001$) threshold concentration of K⁺ to trigger SD in alpha2+/- mice ($13,03 \pm 1,24$ mmol/l, $n = 18$) compared to their wild-type littermates ($14,92 \pm 1,59$ mmol/l, $n = 23$). This fact was reflected by a shortening of the wash-in time needed to induce SD. No significant reduction in threshold concentration was found in alpha1+/- or alpha3+/- mice compared to their wild-type littermates indicating that the observed effect in the alpha2 group is specific for this isoform.

Conclusions: The present results bolster the notion that different catalytic Na,K-ATPase alpha isoforms have distinct functional properties and substantiates the hypothesis that functional haploinsufficiency may underlie the increased susceptibility to SD in FHM2.

Integrating spreading depolarizations in the circuit dysfunction of migraine

KC Brennan

Department of Neurology, University of Utah, Salt Lake City, UT, USA

Spreading depolarizations (SD) are thought to underlie the migraine aura, and thus constitute arguably the earliest physiologically accessible element of the migraine attack. SD have also been implicated in the generation of a pain percept, via their effects on the trigeminal nucleus caudalis (TNC). However migraine is more than the aura, and it is more than just pain – a migraine attack involves multisensory amplifications that cannot be explained only by SD activation of the TNC. We will discuss what is known of the circuit architecture and dysfunction of migraine, as well as what we can learn from other branches of sensory and pain neuroscience. The goal is to understand the potential roles of SD in the migraine network, and ultimately to generate testable hypotheses about how SD can cause the widespread effects that constitute the migraine attack.

Functional and structural brain alterations in chronic migraine – implications for a progressive disease?

Lars Neeb¹, Kaili Bastian^{1,2}, Katharina Kramer^{1,2}, Carsten Finke^{1,3}, Kersten Villringer², Hunter C. Gits², Heike Israel-Willner¹, Uwe Reuter¹, Jochen B. Fiebach²

¹ Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany; ² Center for Stroke Research Berlin, Charité – Universitätsmedizin Berlin, Berlin, Germany; ³ Berlin School of Mind and Brain, Humboldt-Universität zu Berlin, Berlin, Germany

Background: Episodic migraine (EM) may evolve slowly to chronic migraine (CM). Modern neuroimaging techniques such as Voxel based morphometry (VBM), diffusion tensor imaging (DTI) and BOLD resting state functional magnetic resonance imaging (rs-fMRI) have demonstrated mainly in episodic migraineurs structural and functional changes in brain regions involved in pain processing. Some of these changes correlated with migraine duration and attack frequency suggesting that migraine is a progressive disorder of the brain with proceeding alterations of the brain. To assess this concept we evaluated possible structural and functional brain alterations in patients with CM and EM compared to healthy controls.

Methods: Individually, age- and sex-matched subjects with CM without aura, EM without aura, and healthy controls (n=21 per group) underwent conventional head magnetic resonance imaging, DTI, VBM and BOLD rs fMRI imaging in a 3T MRI scanner during the interictal period. DTI data were analyzed using a tract-based spatial statistics approach. Functional connectivity in resting state networks was assessed using an independent component analysis and dual regression approach. Imaging modalities were compared between subjects with CM and EM, CM and controls, EM and controls, as well as between all subjects with migraine (EM + CM) and controls. Additionally, correlation of imaging and clinical parameters such as headache frequency was assessed.

Results: We found an increase of gray matter volume (GMV) in amygdala and putamen in CM compared to controls. Headache frequency in all migraineurs correlated positively with GMV in putamen, frontal and temporal gyrus and negatively in left cuneus. We did not find any statistically significant difference in DTI-derived parameters in group wise comparison and in regression analysis. Rs-fMRI revealed alterations of functional connectivity in regions of the frontoparietal and salience network in CM compared to controls.

Conclusion: CM is associated with altered functional connectivity and structural gray matter but no white matter changes in brain regions involved in pain processing but also in affective and cognitive aspects of pain. These findings and correlation of imaging alterations with clinical parameters of disease severity assessed in EM and CM underline the assumption that chronic pain alters the plasticity in the central nervous system.

The earliest neuronal responses to hypoxia in the neocortical circuit are glutamate-dependent.

Omer Revah¹, Ilya Fleidervish² and Michael Gutnick¹

¹*The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel;*

²*Department of Physiology and Cell Biology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.*

Soon after exposure to hypoxia or ischemia, neurons in cortical tissues undergo massive anoxic depolarization (AD). This precipitous event is preceded by more subtle neuronal changes, including enhanced excitatory and inhibitory synaptic transmitter release. Here, we have used patch-in-slice techniques to identify the earliest effects of acute hypoxia on the synaptic and intrinsic properties of Layer 5 neurons, to determine their time course and to evaluate the role of glutamate receptors in their generation. Coronal slices of mouse somatosensory cortex were maintained at 36°C in an interface chamber and challenged with episodes of hypoxia. In recordings with cell-attached electrodes, the open probability of Ca²⁺-dependent BK channels began to increase within seconds of hypoxia onset, indicating a sharp rise in [Ca²⁺]_i just beneath the membrane. By using a high concentration of K⁺ in the pipette, we simultaneously monitored the membrane potential and showed that the [Ca²⁺]_i rise was not associated with membrane depolarization. The earliest hypoxia-induced synaptic disturbance was a marked increase in the frequency of sPSCs, which also began soon after the removal of oxygen and long before AD.

This synaptic effect was accompanied by depletion of the readily releasable transmitter pools, as demonstrated by a decreased response to hyperosmotic solutions. The early [Ca²⁺]_i rise, the early increase in transmitter release and the subsequent AD itself were all prevented by bathing in a cocktail containing blockers of ionotropic glutamate receptors. We found no evidence for involvement of pannexin hemichannels or TRPM7 channels in the early responses to hypoxia in this experimental preparation. Our data indicate that the earliest cellular consequences of cortical hypoxia are triggered by activation of glutamate-gated channels.

Functional impairment of coding capabilities of neocortical neurons that have survived anoxic depolarization.

Omer Revah¹, Ohad Stoler², Andreas Neef³, Ilya Fleidervish² and Michael Gutnick¹

¹*The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel;*
²*Department of Physiology and Cell Biology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel;* ³*Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany*

Neocortical neurons that recover from an episode of anoxic depolarization (AD) are normal in terms of their passive and active electrophysiological parameters. However, little is known about their functional characteristics within the framework of the neocortical circuit. Here, we used neocortical brain slices to examine a new parameter, the dynamic range, which assesses the surviving neuron's encoding capabilities. We show that in neurons that have recovered from two prior periods of AD, the ability to track subtle, synchronized, high-frequency inputs, and thus fire action potentials that are precisely timed relative to the firing of neighboring cells within the cortical circuit, is compromised. This injury, which is still present up to 8 hours after the anoxic incident, is not directly due to hypoxia but rather is associated with the AD itself, since it does not occur in tissue that has been exposed to long periods of hypoxia in the presence of blockers of ionotropic glutamate receptors, which prevent the early Ca^{2+} influx, enhanced synaptic transmitter release, and massive depolarization. Theoretical studies have shown that the ability of a neuron to track high frequencies is a function of the mechanism that generates action potentials at their point of initiation, which, in the case of neocortical neurons, is the axon initial segment. This area of the proximal axon has a highly specialized cytoskeletal structure, and is characterized by prominent immunocytochemical staining for Ankyrin G. We found that the initial segments of neurons that have recovered from AD do not stain for Ankyrin G. Our findings suggest that the high levels of intracellular Ca^{2+} which accompany AD activate proteases that disrupt the structural organization of the axon initial segment and thereby compromise the coding capabilities of neocortical neurons.

Spreading depolarization-induced disruption of dendritic ultrastructure in the murine neocortex revealed by quantitative serial section electron microscopy.

Ioulia V. Fomitcheva¹, Jeremy Sword², Deborah Croom², Sergei A. Kirou^{1,2}

¹Department of Neurosurgery & ²Brain and Behavior Discovery Institute, Medical College of Georgia at Augusta University, Augusta, Georgia 30912, USA

Spreading depolarization (SD) causes rapid neuronal swelling and dendritic beading with spine loss representing acute damage to synaptic circuitry. Yet, very little is known about the immediate impact of SD on synaptic circuits at the ultrastructural level. Urethane-anesthetized mice of the B6.Cg-Tg(Thy1-EGFP)Mjrs/J strain expressing EGFP in a fraction of pyramidal neurons underwent a craniotomy over the sensorimotor cortex and *in vivo* 2-photon microscopy was used to assess dendritic integrity. Transient global cerebral ischemia was induced on the microscope stage by bilateral common carotid artery occlusion (BCCAO) achieved by tensioning sutures looped around each CCA. Controlled reperfusion was accomplished by relieving the tension of the sutures as soon as the SD was recorded with a glass microelectrode at the site of imaged dendrites. Ischemia during BCCAO and the return of blood flow during reperfusion were verified by laser speckle imaging. Somatosensory stimulus evoked intrinsic optical signal imaging (IOS) was employed to monitor loss and recovery of cortical circuit function during ischemia and reperfusion. As expected, BCCAO-induced SD invariably beaded dendrites, but dendrites recovered after reperfusion accompanied by the return of IOS maps. After confirmation of the intact dendritic structure in sham-operated mice (n=3), or SD-induced dendritic beading after BCCAO (n=3), as well as dendritic recovery after reperfusion (n=3), mice were perfusion-fixed through the heart with mixed aldehydes and the brain was processed for serial section electron microscopy. Three-dimensional reconstructions from sham-operated mice revealed intact dendrites with spines and healthy synapses. Dendritic cytoplasm contained intact microtubules, tubular mitochondria, and smooth endoplasmic reticulum (SER). Dendrites disrupted by SD in mice subjected to BCCAO were beaded and swollen with watery cytoplasm and disordered microtubules. Mitochondria had blebby appearance with swollen segments interconnected by thin segments indicating the beginning of fragmentation. Several dendritic beads contained swollen cisterns of SER. Many spines were collapsed but still attached to the presynaptic axonal boutons. The cytoplasm of recuperated dendrites

after reperfusion contained arrays of microtubules, tubular mitochondria, and recovered cisterns of SER. All spines on recuperated dendrites had synapses. Our findings indicate that even in tissue with severe energy deficits as during global ischemia, SD-inflicted dendritic injury is reversible if blood flow can be rapidly restored immediately after SD onset.

Supported by the NIH Grant NS083858.

Recurrent fission-fusion of neuronal endoplasmic reticulum by synaptic excitation in physiology and in CSD.

Krzysztof Kucharz, Martin Lauritzen

Department of Neuroscience and Pharmacology, University of Copenhagen, 2200 Copenhagen N, Denmark

BACKGROUND: The neuronal endoplasmic reticulum (ER) is the major intracellular Ca^{2+} store crucial for modulation of neurotransmission and synaptic plasticity. The ER maintains structural continuity through all neuronal compartments, which allows long-distance diffusion of intracellular Ca^{2+} , protein trafficking and synapse-to-soma bidirectional signaling. The fragmentation of neuronal ER is regarded as a hallmark of a cell death. Here, we provide the first longitudinal assessment of ER in the living animal brain. We show that neurons have innate ability to reversibly and recurrently alter ER continuity in response to synaptic input in physiology and in pathology of during cortical spreading depolarization (CSD).

METHODS: We used *in vivo* 2-photon microscopy in transgenic mice expressing soluble EGFP diffusing within neuronal ER lumen. The ER continuity was monitored using developed real-time profile plot variance analysis and with fluorescence recovery after photobleaching. Intracellular Ca^{2+} was monitored with a red-shifted Ca^{2+} indicator. Simultaneously with imaging we performed *in vivo* electrophysiological recordings of direct current (DC) and local field potentials (LFPs; electrocorticogram, ECoG).

RESULTS: In basal state the neuronal ER was continuous. We found that somatosensory stimulation induced rapid (<15 s) loss of continuity between spine and dendritic ER. This effect was transient, and spine and dendritic ER regained continuity within 5 minutes post-stimulation. In CSD, however, neuronal ER underwent massive widespread fragmentation (=fission) into numerous, optically isolated structures. We characterized spatio-temporal characteristics of the ER fission-fusion in relation to CSD wave and show that ER fission can occur locally in neurons, spread along the dendrites towards the soma and is preceded and proportional to intracellular Ca^{2+} increase. With DC return to baseline (neuronal repolarization), the ER gradually regained its continuity, and the cycles of ER fission-fusion could occur repeatedly with consecutive CSD events.

Compared to physiology, the ER fusion in pathology lasted longer and neurons analyzed after 5 min post-CSD still exhibited ongoing fusion. Interestingly, the degree of loss and

regain of ER continuity in CSD mirrored the degree of loss and regain of neuronal activity (LFPs/ECoG). We determined that both, in physiology and in CSD, the ER fission-fusion depended on Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) activation downstream of NMDA receptor (NMDAR) stimulation. Lastly, impeding ER fission by CaMKII inhibition in CSD coincided with amelioration of neuronal silencing following DC shift.

SUMMARY: Our functional assays indicate that ER fission-fusion may be a previously unknown mechanism that has divergent consequences for regulation of synaptic signaling in physiology and disease. We suggest that modulation of ER fission-fusion may potentially help to prevent deterioration of neuronal function following transient brain depolarizations.

Irradiation of abnormal activity across multiple networks from discrete foci undergoing spreading depolarization or epileptic activity in rodents.

Daniel Torres, Tania Ortuño, Julia Makarova and Óscar Herreras

Dpt. Of Translational Neuroscience, Cajal Institute - CSIC, Spain

Different types of brain insults entail time evolving secondary lesions in sites remote to the primary damage, a phenomenon known as diaschisis. The precise mechanisms underlying such lesions are not known. It has been proposed that the damage may be relayed through vascular or neural pathways. While the most severely affected regions can be traced using imaging techniques, these are insufficient to detect subliminal structural or activity changes that may account for a variety of neurological sequelae. We assessed the applicability of recent developments in the recording and analysis of intracerebral local field potentials (LFPs) for the study of baseline activity in multiple brain structures and the identification of the particular connecting pathways involved. The main technique involves the recording of LFPs with high density linear arrays for the gathering of spatial profiles of LFPs and the extraction of pathway-specific generators of activity through spatial discrimination analyses. The so obtained LFP-generators allow the characterization of normal and abnormal ranges of activity in specific pathways connecting neuron populations/structures.

We used two models of focal abnormal activity in anesthetized rats, (1) the generation of spreading depolarization (SD) waves in the CA1 region of the hippocampus, and (2) an epileptic focus elicited in the CA3 region through local disinhibition. Care was taken to assess that SD waves or GABA blockers do not invade other regions. We found that during the spread of unilateral SDs in the CA1, aberrant epileptoid activity appears in the upstream CA3 region and notable changes of activity were also observed in other mono or multisynaptically connected nearby and remote populations in the hippocampus and neocortex. These changes may outlast the duration of SD waves. Such remote LFP generators displayed distinct temporal patterns, and their relative variance is modified in a complex manner. When epileptic activity was chemically ignited in the CA3, sustained alterations of spike rate and LFP activity were detected in the thalamus (VL and VPL), and multiple cortical areas. Lidocaine blockade of activity in interposed regions modified but did not reverse completely the remote alterations. These findings indicate that spatially restricted mild dysfunction may irradiate abnormal synaptic activity to several interlaced networks and promote transient and lasting malfunctioning in distant brain regions through both hypo and hyperactivity.

Epileptiform activity in the blood-brain barrier disrupted hippocampus is associated with altered network oscillations and synaptic plasticity

Kristina Lippmann^{1,2}, Lyn Kamintsky³, Soo Young Kim⁴, Svetlana Lublinsky³, Ofer Prager³, Julia Friedericke Nichtweiß¹, Seda Salar^{1,5}, Daniela Kaufer⁴, Uwe Heinemann^{6,7,*} and Alon Friedman^{3,8,*}

*¹Institute of Neurophysiology, Charité – University Medicine Berlin, Berlin, Germany; ²Carl-Ludwig-Institute for Physiology, Leipzig University, Leipzig, Germany; ³Departments of Physiology and Cell Biology, Cognitive and Brain Sciences, The Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer-Sheva, Israel; ⁴Helen Wills Neuroscience Institute and the Department of Integrative Biology, University of California, Berkeley, CA, USA; ⁵The Saul Corey Department of Neurology, Albert Einstein College of Medicine, New York City; ⁶Neuroscience Research Center, Berlin, Germany; ⁷Excellence Cluster NeuroCure, Berlin, Germany; ⁸Department of Medical Neuroscience, Faculty of Medicine, Dalhousie University, Halifax, Canada; *These authors contributed equally to this work.*

Acquired epilepsies in the elderly believed to result from brain microvascular injuries and blood-brain barrier (BBB)-disruption including stroke, traumatic injury and vascular dementia. Post-stroke epilepsy is associated with cognitive decline and poor neurological outcome. Seizures and spreading depolarization (SDs) arise as characteristic activity from ischemic lesions but may also contribute to lesion progression and reorganization of the adjacent neural network and are therefore electrophysiological hallmarks of the pathological process. As an important structure for memory consolidation but also as a region prone to epileptogenesis, the hippocampus is an attractive structure for investigating mechanisms underlying the pathophysiology of microvasculopathy-associated changes in synaptic plasticity and neural network activity.

Cortical photothrombosis, induced by Rose bengal in Wistar rats, led to early peri-ischemic BBB-dysfunction in the adjacent hippocampus and to increased intracranial pressure preceding vasogenic edema. Intrahippocampal recordings of field potentials revealed electrographic seizures within the first week in two thirds of animals. Predominantly seizing animals displayed an increase in theta and reduction in gamma frequency bands suggesting disturbed inhibitory activity. Synaptic interactions and plasticity were studied in parasagittal hippocampal slices at 24 hrs and 7 days post-stroke. Field potential recordings in CA1 uncovered multiple population spikes, epileptiform episodes and SDs at 24 hrs declining at day 7. Input-output analysis revealed that fEPSP-spike coupling was significantly enhanced at 7 days. Feedback and

feedforward inhibition were diminished over the first week. Slices generating epileptiform activity at 7 days revealed impaired bidirectional long-term plasticity following high and low frequency stimulation protocols. Supporting these findings, microarray and PCR data confirmed changes in expression of astrocyte-related genes and suggested downregulation in expression of GABA_A-receptor subunits.

In conclusion, BBB dysfunction in the peri-infarct hippocampus is most prominent during the first week and is associated with hyperexcitability, early disinhibition and abnormal synaptic plasticity. Our data suggest that early BBB dysfunction and SDs may be associated with epileptiform activity, excitatory-inhibitory imbalance and pathological long-term plasticity. Together, we suggest that new diagnostic monitoring opportunities in patients at risk for epileptogenesis and cognitive impairment may be warranted, specifically when BBB-dysfunction is observed.

Ictal activity associated with spreading depolarization in the aged rat brain: reality or illusion?

Eszter Farkas, Péter Makra, Gábor Kozák, Ferenc Bari, Ákos Menyhárt

Department of Medical Physics and Informatics, Faculty of Medicine, University of Szeged, Korányi fasor 9, Szeged, Hungary

Although a typical feature of spreading depolarizations (SDs) is the depression of local field potentials (LFPs), ictal activity has been shown to evolve simultaneously with SD in the acutely injured human brain. In our ongoing rat aging studies, we have recognized rhythmic, high amplitude LFP oscillations coupled to SDs in the intact and ischemic cortex, and have set out to investigate this phenomenon comprehensively.

While the SD-related classic LFP depression typically evolved in young rats, rhythmic, high amplitude LFP oscillations (frequency: 0.98 ± 0.19 Hz; max peak: 242 ± 161 μ V) emerged with SDs at ~50 % incidence in old animals (intact: 17/29 SDs; ischemia: 16/30 SDs). Episodes of such spindle- or horizontal sand-glass-shaped events evolved synchronously with the depolarization, plateau and repolarization of SD, and persisted for over a minute in average. Green reflectance image sequences of the cortex unraveled, that SDs with rhythmic LFP oscillations often propagated with an irregular pattern in contrast to the radial propagation common for SDs with classic LFP depression.

As compared with classic LFP depression, SDs with the rhythmic LFP oscillation were associated with a lower mean LFP amplitude (15.36 ± 2.0 vs. 24.91 ± 10.6 μ V) and lower cerebral blood flow (58.3 ± 26 vs. 72.5 ± 29 %) taken prior the onset of each ischemic SD, and lower heart rate (296 ± 27 vs. 308 ± 28 bpm) and respiratory rate (48 ± 3 vs. 63 ± 5 bpm) during SD. The frequency of SD-associated ictal activity in the injured human brain was reported to fall between 0.5-5 Hz (Fabricius et al., 2008; Dreier et al., 2012), which overlaps with the frequency of LFP oscillations detected here. All these evidence inferred that the rhythmic LFP oscillations observed were physiologically relevant.

Before recognizing the LFP phenomenon as ictal activity, the contribution of potential artifacts had to be ruled out. In particular, the possibility of pulse transmission artifact earlier demonstrated to occur in patients (Dreier et al., 2012) was considered and identified only in one animal at 4.8 Hz. Fourier analysis of the blood pressure signal recorded from the femoral artery synchronous with the LFP however revealed that the first, small peak in the blood pressure spectrum coincided with the fundamental peak in

the LFP spectrum during rhythmic LFP oscillations in all cases. This frequency (0.98 ± 0.19 Hz) has been recognized as the respiration rate of the animals.

It appears that the SD-associated rhythmic LFP oscillations we recorded were related to respiration, but it remains to be resolved why they emerged only in old rats, and why they were associated with physiological variables distinct for this subgroup of SDs.

Seizure-induced pericytic injury is associated with neurovascular decoupling and opening of the blood-brain barrier

Luisa Austin Hasam², Vera Wuntke², Ofer Prager¹, Lyn Kamintsky^{1,3}, Karl Schoknecht², Ismini Papageorgiou², Jutta Swolinsky², Valeria Muoio², Uwe Heinemann[†], Alon Friedman^{1,3}, Richard Kovács²

¹Department of Physiology & Cell Biology, the Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer-Sheva, Israel; ²Institute for Neurophysiology, Charité - University Medicine Berlin, Germany; ³Department of Medical Neuroscience, Dalhousie University, Halifax, Canada

Opening of the blood-brain-barrier (BBB), redistribution of pericytes and angiogenesis are common findings in epilepsy, yet the link between seizures and disturbances of the neurovascular unit remains elusive. In the present study we followed successive changes in neurovascular coupling (NVC) during recurrent seizures by recording the neuronal activity, pericytic membrane currents, changes in tissue pO₂ in parallel to monitoring of tightness and motility of capillaries and formation of free radicals in a slice culture model of microvasculature.

Hippocampal capillary network was preserved in culture and NG2 positive pericytes regulated vascular diameter in response to seizures and vasoactive agents. The maintenance of multidrug transporter activity at the BBB was evidenced by the accumulation of dichlorofluorescein (DCF) in the capillary lumen. Whole cell patch clamp recordings of pericytes revealed distinct current patterns upon application of the thromboxane analogue, U46619 and seizures, which contributed to pericytic contraction and elongation. Individual seizures resulted in vasodilation irrespective of the type of the pro-convulsive treatment. However, vasodilatory responses eroded during the course of recurrent seizures in parallel with an increase in fluorescence of the mitochondrially targeted free radical marker, MitoSox. Neurovascular decoupling could not be attributed to impaired neurometabolic coupling, as seizure-induced increases in respiration remained unaltered for recurrent seizures. Remarkably, pericytic rigor and vasoconstriction were accompanied by increased permeability of the BBB. Although seizures enhanced the formation of vasoactive free radicals in the brain parenchyma, the free radical scavenger, TEMPOL could not prevent pericytic injury and vasoconstriction. The present study represents the first time direct observation of a gradually developing neurovascular decoupling and pericytic injury preceding disruption of BBB during recurrent seizures.

Role of capillary pericytes and penetrating arteriole during cortical spreading depression

Lila Khennouf^{1,*}, Bodil Gesslein¹, Alexey Brazhe^{1,2}, Christopher J. Oceau³, Baljit S. Khakh³ and Martin Lauritzen^{1,2,4,*}

¹*Department of Neuroscience and Pharmacology, University of Copenhagen, 2200 Copenhagen N, Denmark;* ²*Department of Biophysics, Faculty of Biology, Moscow State University, 119234 Moscow, Russia;* ³*Departments of Physiology and Neurobiology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095-1751 USA;* ⁴*Department of Clinical Neurophysiology, Glostrup Hospital, 2600 Glostrup, Denmark;*

Cerebral blood is reduced after CSD, along with impaired neurovascular coupling and reduced stimulation-induced neuronal and glial calcium responses (Khennouf et al., 2016). The current study explored the reaction of penetrating arterioles and capillaries to CSD with specific regard to vascular branching order, pericyte location and pericyte calcium responses. Pericytes are specialized vascular cells covering brain capillaries extensively, which may control capillary diameter *in vivo* (Hall et al., 2014). We hypothesized that pericytes play a key role in the long-lasting constriction and impaired neurovascular coupling following CSD.

We used mice expressing the DsRed fluorescent indicator under a pericyte promoter and compared diameter changes of penetrating arterioles and 1st to 3rd order of capillaries in relation to localization of pericyte cell bodies and processes. The vessels were outlined with intravenous injection of FITC dextran, and imaged using two-photon microscopy. Calcium is a marker of activity for actin containing cells, and was used to indicate changes in pericyte activity. In order to monitor calcium changes via two-photon microscopy, we engineered a genetically encoded calcium indicator specific to pericytes. We measured GCaMP6s fluorescence and vessel diameter changes *in vivo* during somatosensory stimulation and CSD in barrel cortex region of mice.

CSD vascular changes were characterized by an initial fast and small constriction, followed by large vasodilation and long-lasting vasoconstriction. During the initial constriction, penetrating arterioles and capillaries both constricted by 8% of the pre-CSD baseline. The vasodilation during CSD was accompanied by a 15 % dilation of penetrating arterioles, while 1st order capillaries dilated by 12%. This dilation was accompanied by a decrease in GCaMP6s fluorescence in pericytes. After CSD, 1st order capillaries constricted by 15%, while penetrating arterioles constricted by 11%, which

may suggest that capillary vasoconstriction is a main factor for the persistent oligemia after CSD. The constriction of capillaries was accompanied by a calcium rise in pericyte cell bodies located on 1st order capillaries. Under control conditions, somatosensory stimulation dilated penetrating arterioles and 1st order capillaries to a larger extent than 2nd and 3rd order capillaries and this was accompanied by a drop in pericyte calcium fluorescence. After CSD, the whisker pad stimulation-induced vascular dilation was reduced for all calluses of vessels, with an average of 2% increase during stimulation accompanied by a smaller drop in pericyte calcium fluorescence than under control conditions. The data show that CSD-evoked vascular changes occur mainly at the level of the penetrating arteriole and 1st order capillaries levels and that pericyte calcium responses contribute to the complex vascular reaction.

Hall, C.N., Reynell, C., Gesslein, B., Hamilton, N.B., Mishra, A., Sutherland, B.A., O'Farrell, F.M., Buchan, A.M., Lauritzen, M., and Attwell, D. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 508, 55-60.

Khenouf, L., Gesslein, B., Lind, B.L., van den Maagdenberg, A.M., and Lauritzen, M. (2016). Activity-dependent calcium, oxygen, and vascular responses in a mouse model of familial hemiplegic migraine type 1. *Annals of neurology* 80, 219-232.

Adenosine A_{2A} receptor activation contributes to vasodilation of cerebral parenchymal arterioles following spreading depolarization

Britta E. Lindquist, C. William Shuttleworth, PhD

Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, New Mexico

Spreading depolarization (SD) is a propagating wave of activation carried through brain tissue by K⁺ and glutamate. SD causes profound metabolic demand, marked by adenosine accumulation, prolonged synaptic depression and species-dependent changes in blood flow. The mechanisms by which tissue recruits blood flow during recovery remain incompletely understood, and might involve activation of adenosine A_{2A} receptors on cerebral blood vessels. Here, we modified an *in vitro* model to evaluate responses of parenchymal arterioles to SD. Acute coronal brain slices (310 μm) were prepared from C57Bl/6 mice, and parenchymal arterioles (PAs) were visualized in cortical regions with infrared differential interference contrast imaging, and pre-constricted with U-46619 (30-300nM), to a physiologic range of basal tone (30-50%). Exogenous adenosine in the range observed in the cortex following SD (20-30 μM) maximally dilated PAs (to 95.2±4.6% passive diameter, n=3), and effects of adenosine were completely reversed by A_{2A} receptor antagonist ZM-241385 500 nM (p<0.05, n=3). In response to SD, induced by a remote focal injection of KCl (1M), PAs demonstrated biphasic vasomotor responses, with initial constriction (net change -15.7±3.9%) occurring 29.0±9.2s after the SD wave-front, followed by prolonged dilation, to 92.0±3.9% passive diameter. The degree of dilation, but not the degree of constriction, was dependent on basal tone (R²=0.49, p<0.05, n=11). Next, we tested the hypothesis that adenosine A_{2A} receptor activation contributed to vasodilation following SD. Pre-incubating slices with ZM-241385 prior to SD did not affect basal tone or initial constriction to SD (basal tone 76.5±5.3 vs 78.2±6.9%, p=0.83, net constriction -19.2±7.2 vs -19.9±4.7%, p=0.93, n=5,5), nor did it prevent vasodilation following SD, (net dilation 22.3±5.1 vs 13.3±4.5%, p=0.18, n=5,5). In the presence of nitric oxide synthase inhibitor L-NNA (100 μM), ZM-241385 significantly reduced vasodilation (net dilation 2.5±6.6 vs 24.0±6.5%, ZM-241385 and L-NNA vs L-NNA alone, n=8,7, p<0.05), without altering basal tone or initial vasoconstriction (p=0.81). These findings suggested that adenosine is present at concentrations sufficient to dilate parenchymal arterioles following SD, but that it is not required as long as the nitric oxide pathway remains intact. Mouse arterioles demonstrated bidirectional diameter changes after SD, with dilation limited by basal tone, suggesting a ceiling effect. The slice preparation is a suitable model for characterizing pharmacology of cerebral vasomotor responses to SD, without confounding effects of drugs on the cardiovascular system.

Tracking membrane potential changes during spreading depolarisations: A case report

Sharon L Jewell^{1,5}, Jordi Serra^{1,2}, Jose-Pedro Lavrador³, Clemens Pahl⁴, Marytn G Boutelle⁵, Anthony J Strong¹.

¹Department of Clinical Neuroscience, King's College London; ²Department of Clinical Neurophysiology, King's College Hospital, London; ³Department of Neurosurgery, King's College Hospital; ⁴Department of Anaesthesiology, King's College Hospital, London, ⁵Department of Bioengineering, Imperial College London.

Introduction: Local injury, poor blood supply and hypoxia can profoundly disrupt the regulation of neuronal membrane potential (V_m) and can render the cortex susceptible to spreading depolarisations (SD); an important contributor to the enlargement of cerebral infarcts. Measuring changes in V_m might therefore provide a means of detecting impending neural damage arising from imminent SD's and may enable us to better understand the pathophysiological consequences of SD on the viability of neurons. Utilising measures of excitability we have recently developed a method to monitor changes in V_m in-vivo. In this case study, we were able to monitor the V_m changes that arose before, during and after SD waves and during ketamine administration.

Case summary: *Background:* A 41yr old male was admitted with a right sided traumatic subdural and subarachnoid haemorrhage. CT scan also showed subfalcine herniation and 14mm midline shift.

Intervention: The patient underwent a fronto-temporal craniectomy. At conclusion, a subdural (6 platinum electrode, exposed \varnothing 2.3mm, 10mm centre-to-centre) and intraparenchymal (8 platinum electrode, \varnothing 1.1mm, 1.3mm long, 2.2mm centre-to-centre, Adtech, USA) array was placed in penumbral tissue. A combined oxygen & temperature (Integra, UK) and an intracranial pressure probe (DePuy Synthes, USA) was also placed.

Monitoring summary: The patient underwent 6 days of monitoring. A total of 88 SDs were recorded. On day 2 a Ketamine intravenous infusion was commenced. Ketamine infusion continued for 38.5hrs. During this time, 1 SD was seen. Upon cessation, 2 further SDs were recorded before monitoring was stopped.

Excitability tracking: Absolute threshold and multi-tracking of the Compound Bundle Action Potential (CBAP) at interstimulus intervals of 20 and 100ms along the recovery cycle was commenced on day 2. Continuous excitability tracking was undertaken for up to 10hours.

Discharge: The patient was discharged home 24 days after admission with no motor or speech deficit and full power in all limbs. He was GCS 15 at discharge.

Conclusion: 1) Distinct profiles of excitability emerge before, during and after SD waves. Excitability tracking could therefore be used as a clinical tool for identifying patients who will benefit from treatment and for identifying the opportune moment for clinical intervention. 2) Ketamine had a profound effect on blocking further passage of SD. 3) Ketamine profoundly altered absolute threshold and post-activation V_m 3) Ketamine did not affect the CBAP amplitude. We therefore believe the CBAP is a directly axo-axonal response. 4) SD appears to infiltrate at least the proximal segments of subcortical projecting axons.

UK Patent Application Number 1609207.4. "Indexing Neuronal Homeostasis in the Central Nervous System".

Effect of reperfusion on the occurrence of spreading depolarizations in a model of focal transient cerebral ischemia in rats.

Nielsen Lagumersindez Denis¹, Clément BRUNNER², Gabriel MONTALDO² and Alan URBAN²

¹Charité-Medical University of Berlin; Department of Neurology with Chair in Experimental Neurology; ²Laboratory of neural circuits, NERF, IMEC, VIB, KU Leuven, Leuven, Belgium

Spreading depolarizations (SDs) are slow self-propagating waves of intense regional depolarization of neuroglial cells causing suppression of electrical activity, accompanied by ionic and metabolic imbalance. In the context of ischemic stroke, several studies have identified a persistent depolarization as a pathological signature of the ischemic core rendering the tissue irreversibly damaged within this territory. Additionally, secondary spontaneous SDs propagating through the adjacent cortex, contribute to a delayed expansion of the infarct region. Altogether, this makes SDs a potential target for achieving neuroprotection. Because SDs are a transient phenomenon, an accurate definition of the anatomical site where they originate or a follow up of their whole propagation trajectory is not always possible with the current imaging techniques available. Preliminary data from our group, combining functional ultrasound imaging (fUSi) and multi-electrodes recording allowed us to map successfully hemodynamic/electric changes in tissue perfusion during ischemia/reperfusion but also to image SDs in a transient distal middle cerebral artery (MCA) occlusion model in rats using a microvascular clip. A few minutes after MCA occlusion subsequent SDs arised in the peri-ischemic tissue and propagated into the adjacent normally-perfused motor cortex from the ipsilateral hemisphere. Immediately before single SDs happened, a transient hypoperfusion was observed in these areas followed up by a large hyperemia (+200% compared to baseline) as indicated by the increase of relative cerebral blood volume (rCBV). fUSi recordings during the reperfusion phase showed a reduced average rate of SDs occurrence, reflecting a probable stabilization of the metabolic/ionic impairment in the penumbra region. Astrocytes in the other hand, can be indirect mediators of the hyperemic response observed upon whisker stimulation and may provide critical metabolic support to other cellular elements of the neurovascular unit for their survival. Whether any of these phenomenon account for the effect observed on SDs and on infarct volume still need to be answer in future experiments. Our findings validate the technical feasibility of fUSi combined with electrical recordings as a promising method to better understand the dynamic of SDs in the context of stroke, migraine and other cerebrovascular diseases.

Influence of tissue metabolic status on Ca²⁺ and glutamate accumulation during spreading depolarization

Katelyn M. Reinhart and C.W. Shuttleworth

Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, NM, USA.

Repetitive waves of spreading depolarization (SD) are now clearly implicated in progression of acute brain injuries, with vulnerability determined by the metabolic competence of brain tissue through which they propagate. We previously reported initial studies of murine brain slices that were metabolically compromised, but fully viable until a SD wave propagated through them (Cosbid 2016). The present report extends these observations to further validate the model, characterize damaging glutamate and Ca²⁺ loading, and test the hypothesis that low concentrations of ketamine can protect vulnerable tissue from SD injury without occluding potentially beneficial NMDAR-dependent plasticity in healthy tissues. KCl microinjection was used to initiate SD in 350µm hippocampal slices and responses were recorded in the CA1 subfield. Neuronal Ca²⁺ was assessed using GCaMP5G, while extracellular glutamate was detected using iGluSnFR. Responses in control conditions were compared with compromised conditions, which were achieved by restricting superfusion to the top surface of slices. In these slices, substrate availability was reduced but perfusion rate was maintained at 2 ml/min. NADH and O₂ measurements confirmed basal metabolic compromise, and assessment of evoked excitatory postsynaptic potentials (epsp; paired pulse facilitation) was consistent with reduced initial release probability, secondary to metabolic status at baseline. Metabolically compromised tissues remained viable for hours, in the absence of an SD challenge. 100% of control slices recovered fully after SD, as assessed by 1) intrinsic optical signals, 2) recovery of epsps, and 3) ability to generate a subsequent SD. In contrast, almost all metabolically compromised slices were not viable after the passage of a single SD. In vulnerable slices, DC shifts were longer and matched by significantly longer extracellular glutamate transients ($\tau \sim 40s$ vs. $\sim 25s$; compromised vs. control). Likewise, Ca²⁺ loading in both somatic and dendritic compartments was markedly enhanced in compromised tissues. A low concentration of ketamine that did not prevent SD (30µM), normalized Ca²⁺ transients in both soma and dendrites of compromised tissues, and slices demonstrated recovery of epsps and ability to generate repetitive SDs. Finally, a post-hoc analysis showed that 11/16 preparations exhibited a 5-98% increase in postsynaptic potential amplitude after the first SD. These results further demonstrate the influence of metabolic status on both glutamate and Ca²⁺ accumulation during SD, and support potential utility of interventions that do not block, but reduce deleterious consequences of SD.

Supported by NIH grants NS51288, & T32 HL007736

Contribution of prostanoid signaling to the evolution of spreading depolarization and the associated cerebral blood flow response

Dániel Péter Varga, Ákos Menyhárt, Tamás Puskás, Péter Hertelendy, Dániel Zölei-Szénási, Ferenc Bari, Eszter Farkas

Department of Medical Physics and Informatics, Faculty of Medicine & Faculty of Science and Informatics, University of Szeged, H-6720 Szeged, Korányi fasor 9, Hungary.

Recurrent spreading depolarizations (SDs) are associated with atypical hemodynamic response in the ischemic cortex, which is thought to contribute to the expansion of the ischemic core by decreasing cerebral blood flow (CBF) and increasing the energy demand of the cells. The significance of prostanoid signaling in neurovascular coupling during somatosensory stimulation is increasingly more appreciated, yet its involvement in mediating the CBF response to SD has remained inconclusive.

Selective cyclooxygenase (COX) enzyme inhibitors (SC-560, NS-398) or an antagonist (L161,982) of the EP4 type prostaglandin E2 receptor were applied topically to a cranial window over the parietal cortex of isoflurane-anesthetized Sprague-Dawley rats ($n = 60$). Global forebrain ischemia was induced by common carotid artery occlusion in half of the animals. SDs were triggered by topical application of 1M KCl. SD occurrence was confirmed by the acquisition of DC potential, and CBF variations were recorded by laser-Doppler flowmetry.

EP4 receptor antagonism significantly decreased the SD-coupled peak hyperemia (50 ± 21 vs. $76 \pm 37\%$) and augmented post-SD oligemia (58 ± 13 vs. $40 \pm 14\%$) in the intact cortex, whereas the selective inhibition of COX-1 and COX-2 enzymes exerted no significant effect. In the ischemic cortex, our study revealed a considerable reduction of the distinct elements of the CBF response to SD, with no detectable impact of COX enzyme inhibition or EP4 receptor blockade. Interestingly, inhibition of COX-1 or the blockade of EP4 receptors remarkably delayed repolarization after SD (154 ± 58 and 120 ± 79 , vs. 42 ± 14 s) in the ischemic brain, while the inhibition of COX-2 remained ineffective in this respect.

Our data suggest, that activation of EP4 receptors initiates vasodilation in response to SD in the intact brain. In addition, COX-1 derived prostanoids – together with the activation of EP4 receptors– shorten SD duration in the acute phase of ischemia. These significant observations may initiate a new line of investigation to dissect specific components of prostanoid signaling that may play a defining role in sustaining or aborting SD in the ischemic nervous tissue.

Functional MRI for Assessment of the Default Mode Network in Acute Brain Injury

Daniel Kondziella¹, Patrick Fisher², Vibeke Andrée Larsen³, John Hauerberg⁴, Martin Fabricius⁵, Kirsten Møller⁶, Gitte Moos Knudsen²

¹*Departments of Neurology*, ³*Neuroradiology*, ⁴*Neurosurgery*, ⁵*Clinical Neurophysiology*, and ⁶*Neuroanaesthesiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark*; ²*Neurobiology Research Unit, Copenhagen University Hospital and Center for Integrated Molecular Brain Imaging, Copenhagen, Denmark*

Background: Resting state functional Magnetic Resonance Imaging (fMRI) can be used to evaluate the default mode network (DMN) in patients with chronic brain injury. Preservation of the DMN is associated with higher levels of consciousness. In acute brain injury, however, assessment of the DMN is much more challenging for various reasons, including cardiovascular vulnerability, intravenous sedation and artificial ventilation. In this feasibility study, we aimed at investigating the DMN in patients with acute brain injury admitted to our neurocritical care unit.

Methods: Patients fulfilled inclusion criteria if they had an acute brain injury (≤ 21 days from onset) of traumatic (TBI) or non-traumatic origin, requiring admittance to the neurocritical care unit and ventilatory support but no or only minimal sedation. Patients were diagnosed as being in 1) coma, 2) a vegetative state (VS)/unresponsive wakefulness syndrome (UWS), 3) a minimal conscious state minus or plus (MCS-/+,) or 4) a conscious state (CS) according to established criteria (The Multi-Society Task Force on PVS). Resting state fMRI was performed using a systems-level approach as described earlier. The DMN was defined as encompassing the posterior cingulate cortex/precuneus, medial prefrontal cortex, lateral parietal cortex, inferior temporal cortex, and thalamus bilaterally. The DMN was classified dichotomously into “well-preserved” and “not definitely present”. Levels of consciousness and modified Rankin Scale (mRS) scores were re-assessed at 3 months follow-up.

Results: Between October 2015 and August 2016 we evaluated a convenience sample of 7 patients with acute brain injury (4 females; median age 37 years (range 14-71 years); 1 TBI, 6 non-TBI (basilar artery thrombosis, autoimmune encephalitis, obstructive hydrocephalus, SAH with secondary thalamic infarctions, drowning followed by cardiac arrest, and cardiac arrest following pulmonary embolism). Neurological presentation at fMRI was as follows: 2 coma, 1 VS/UWS, 3 MCS -, and 1 MCS +; and clinical outcome at 3

months follow-up: 1 death, 1 VS/UWS, 1 MCS –, and 4 CS (1 mRS 0; 2 mRS 4; 1 mRS 5). Well-preserved DMNs were noticed in 4 out of 7 patients (1 MCS –, 3 CS at follow-up).

Discussion: This study shows that it is feasible to conduct resting state fMRI to assess the DMN in patients with acute brain injury requiring artificial ventilation. However, the level of sedation during fMRI scans is an important confounder that must be carefully monitored. Although preliminary data, all patients with a preserved DMN regained consciousness levels at follow-up compatible with MCS – or better.

Putative spreading depolarizations after diffuse hypoxic-ischemic brain injury identified with invasive continuous cerebral perfusion monitoring

Ramani Balu, MD/PhD¹; Swarna Rajagopalan, MD¹; Wesley Baker PhD^{2,3}; Benjamin Abella, MD⁴; W. Andrew Kofke, MD²

¹Department of Neurology; ²Department of Anesthesiology and Critical Care Medicine; ³Department of Physics; ⁴Department of Emergency Medicine, University of Pennsylvania

Diffuse hypoxic ischemic brain injury (HIBI) is a major cause of death and disability worldwide. Injury mechanisms after global hypoperfusion are incompletely understood but likely involve dysregulated tissue perfusion, excitotoxicity, impaired oxygen and glucose utilization, and inflammation. Spreading depolarizations (SDs) link these varied mechanisms together, and recent studies show that SDs frequently occur after ischemic stroke, subarachnoid hemorrhage (SAH), intracerebral hemorrhage, and traumatic brain injury (TBI). Global anoxia and hypoperfusion reliably trigger SDs in rodent models; however, to date they have not been identified in humans with HIBI. Using invasive real-time measurements of cerebral perfusion, we identified multiple hemodynamic events that strongly suggest that SDs occur in this patient population as well. From October 2014 to January 2017, we cared for 9 patients with HIBI where invasive multimodality neuromonitoring was used to guide therapy. All patients had a bundle of invasive monitors placed into brain parenchyma that included (1) an ICP monitor (Camino, Integra), (2) Clark electrode (Licox, Integra) and (3) thermodilution blood flow probe (Bowman Perfusion Monitor, Hemedex). In 5 of the 12 patients, cerebral microdialysis was also performed. 6 of the 9 patients had prominent low frequency (0.01-0.1 Hz) vascular fluctuations (LF-VF) interspersed with prolong periods of LF-VF suppression that were strikingly similar to SD induced suppressions of LF-VF reported in patients with SAH. LF-VF suppression correlated with the degree of brain tissue hypoxia and metabolic crisis (cerebral glucose < 0.7 mmol/L, lactate/pyruvate ratio > 40). In addition, 2 patients had frequent transient drops in cerebral perfusion of > 20% below baseline similar to SD evoked inverse hemodynamic responses identified after TBI and SAH. Taken together, we propose that these characteristic hemodynamic findings represent SD induced spreading ischemia waves triggered by HIBI. Further work using invasive electrophysiological measurements is required to validate the presence of SDs in this patient population.

Substance P is able to elicit cortical spreading depolarization (CSD) in adult rats – a mechanism responsible for aggravation of cortical damage after brain injury or stroke?

F. Richter¹, J. Leuchtweis¹, A. Eitner¹, A. Lehmenkühler², H.-G. Schaible¹

¹Institute of Physiology, FSU Jena, D-07740 Jena, Germany; ²Pain Institute & Center for Medical Education, D-40217 Düsseldorf, Germany

Data from the literature indicate that substance P is released in the brain after traumatic brain injury [1] or stroke [2]. It is assumed that substance P (SP) critically contributes to secondary damage in the brain, e.g. by inducing vasogenic edema [3]. In an earlier study we have shown that 10^{-5} M SP induced a significant plasma extravasation in rat dura mater [4]. The particular pathophysiological processes through which SP induces secondary damage have not been clarified yet. In this study we tested, whether SP induces CSD, an energy consuming process, particularly in a previously damaged brain.

We recorded in spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) CSD in cerebral cortex with two pairs of glass micropipettes (distance 5-6 mm) at depths of 400 and 1200 μ m in two areas of the cerebral cortex, separated by a wall. In one area, CSD was elicited by a microinjection of 1 M KCl (tip diameter 5 μ m, 100 kPa, 300 ms–1 s) into the grey matter at intervals of 30 min. 100 μ l of 10^{-5} M SP were applied topically to the remote cortical surface and left there. In another group of rats, we performed microinjections of 10^{-5} M SP into the grey matter at different depths starting from 1200 μ m up to 200 μ m in order to evoke CSD by this stimulus pulse.

In all rats tested, a pulse of KCl elicited a single propagating CSD. The topical application of SP to the brain surface induced a series of CSD (3-7 within the first 30 min of application) that originated in the SP-treated area. Amplitudes of CSD did not differ from those elicited by KCl, and 87 % of the CSD propagated into the untreated cortical area. The application of SP did not influence the CSD waves in the untreated area. In the treated area, however, an increase in the amplitudes of CSD was seen (400 μ m depth: control 21.3 ± 3.0 mV, after two hours 23.2 ± 4.8 mV; 1200 μ m depth: control 21.7 ± 1.4 mV, after two hours 27.4 ± 4.0 mV). The propagation velocity of CSD elicited by KCl was not changed by the application of SP (control 2.7 ± 0.6 cm/min, after two hours 2.4 ± 1.0 cm/min). Interestingly, microinjections of 10^{-5} M SP with pulses of 300 ms–1 s in cortical depths between 600 and 1200 μ m failed to elicit CSD.

Our results confirm that SP is a candidate to elicit CSD independently from other depolarizing agents. Long-lasting application of SP increases CSD amplitudes indicating a stronger depolarization. Therefore SP-induced CSD might contribute to secondary brain damage besides the other pathophysiological effects of SP.

[1] Corrigan et al., J Neuroinflammation. 2016;13(1):264.

[2] Turner RJ, Vink R., Brain Sci. 2013;3(1):123-42.

[3] Donkin et al., J Cereb Blood Flow Metab. 2009;29(8):1388-98.

[4] Ebersberger et al., Ann Neurol. 2001;49(1):7-13.

Tracking membrane potential changes in the in-vivo human brain *Part I: Monitoring changes in baseline membrane potential and during the recovery cycle of excitability.*

Sharon L. Jewell^{1,5}, Jordi Serra^{1,2}, Hugh Bostock³, Jose-Pedro Lavrador⁴, Marytn G Boutelle⁵, Clemens Pahl⁶, Anthony J Strong¹.

¹Department of Clinical Neuroscience, King's College London; ²Department of Clinical Neurophysiology, King's College Hospital, London; ³Institute of Neurology, University College London; ⁴Department of Neurosurgery, King's College Hospital, ⁵Department of Bioengineering, Imperial College London; ⁶Department of Anaesthesiology, King's College Hospital, London.

Background: Spreading Depolarisations (SD) exist along a continuum ranging from those that play a causal role in expanding infarct volume through to those whose clinical relevance is less clear. The critical variable in determining the pathophysiological consequence of SD is not the number of events per se, but the time spent in a depolarised state. A measurement of membrane potential (V_m) might therefore provide a means of discriminating between SD's that are harmful and those which may be more innocuous. The problem is however, that V_m is conventionally studied with voltage clamp techniques - an approach that is unfeasible in the in-vivo human state. As such, our challenge has been to develop a method to directly interrogate V_m in the in-vivo human brain. One way to achieve this is to probe neuronal excitability.

Objective: To establish the feasibility of excitability tracking as a method to monitor fluctuations in neuronal V_m in-vivo, in the injured human brain.

Methods: *Set-up:* Eleven brain injured patients were recruited. Intraparenchymal (8 platinum electrodes, \emptyset 1.1mm, 1.3mm long, 2.2mm centre-to-centre, Adtech, USA) arrays were placed at conclusion of surgery in 7/11 and via a bolt (Hemedex) in 4/11.

Response acquisition: Responses were acquired using Qtrac (©Institute of Neurology) to control delivery of stimuli via a human-proof constant-current stimulator (DS5; Digitimer, UK). Parameters were kept within safe $\mu\text{C}/\text{cm}^2/\text{phase}$ limits. Signals were band-pass filtered at 5Hz-10kHz and digitized at 50kHz.

Stimulus-response: A response of target amplitude (TA) was set by first recording a stimulus-response curve.

Threshold tracking: The stimulus required to maintain the response at constant amplitude was calculated by comparing the TA with that of the response just recorded.

Recovery Cycle of excitability (RC): The RC was obtained by comparing the stimulus required to evoke a response to a single pulse with the stimulus required to evoke the same response after a sequence of afterpotentials were set in motion by a previous pulse given at a series of pre-defined interstimulus intervals.

Results: In 11/11 subjects, stable compound responses with suitable stimulus-response functions were recorded. As no such response, has previously been described, we have designated them Compound Bundle Action Potential (CBAP). Threshold tracking was achieved for up to 10 hours. In 6/11 subjects, full RCs were obtained.

Conclusion: Excitability tracking is a feasible, reliable and safe method to monitor V_m changes in the in-vivo human brain. To our knowledge, this is the first time that RCs have been recorded and V_m changes have ever been tracked in the in-vivo human brain.

UK Patent Application Number 1609207.4. "Indexing Neuronal Homeostasis in the Central Nervous System".

Tracking membrane potential changes in the in-vivo human brain *Part II: Monitoring changes in post-activation afterpotentials.*

Sharon L Jewell^{1,5}, Jordi Serra^{1,2}, Hugh Bostock³, Jose-Pedro Lavrador⁴, Marytn G Boutelle⁵, Clemens Pahl⁶, Anthony J Strong¹.

¹Department of Clinical Neuroscience, King's College London; ²Department of Clinical Neurophysiology, King's College Hospital, London; ³Institute of Neurology, University College London; ⁴Department of Neurosurgery, King's College Hospital; ⁵Department of Bioengineering, Imperial College London; ⁶Department of Anaesthesiology, King's College Hospital, London.

Background: Following an action potential, there is a stereotyped sequence of afterpotentials that give rise to a series of excitability changes known as the Recovery Cycle (RC). During the spike, transient Na⁺ channels inactivate and then gradually recover, giving rise to a period of refractoriness. Next follows a depolarising afterpotential resulting from a passive capacitative discharge of the internodal membrane giving rise to a period of superexcitability. Finally, slow K⁺ channels are activated resulting in an after-hyperpolarisation giving rise to a period of late subexcitability. Na⁺ and K⁺ ion channel function, transmembrane ion gradients and pump activation determine the duration and magnitude of each of the afterpotentials. Therefore, by monitoring alterations in the phases of the RC, it is possible to gain insight into the biophysical changes that arise ahead of, during and after spreading depolarisation waves.

Objective: To establish the feasibility of excitability tracking as a method to monitor fluctuations in post-activation afterpotentials in-vivo, in the injured human brain.

Methods: *Set-up & Response acquisition:* As described in Part I.

Recovery Cycle of excitability: The RC is obtained by comparing the threshold current required to evoke a response to a single stimulus (absolute threshold – described Part I) with the threshold current required to evoke the same response after the RC has been set in motion by a previous 'conditioning' pulse given at a series of pre-defined interstimulus intervals (ISI).

Continuous multi-tracking: Since neurons are rarely in a steady state and repeatedly recording the RC takes time, 'multi-tracking' a select number of phases of interest along the RC provides a continuous measure of changes in membrane potential V_m together with information regarding possible mechanisms. Pairs of stimuli with ISIs of 20 and 100ms were delivered at 0.05Hz.

Results: In 6/11 subjects, full RCs were obtained. In 11/11 subjects, continuous multi-tracking at ISIs of 20 and 100ms was undertaken for up to 10 hours.

Conclusion: Excitability tracking is a feasible, reliable and safe method to monitor changes in post-activation afterpotentials in the in-vivo human brain. To the best of our knowledge, this is the first time that post-activation afterpotential changes have ever been tracked continuously online and real-time in the in-vivo human brain.

UK Patent Application Number 1609207.4. "Indexing Neuronal Homeostasis in the Central Nervous System".

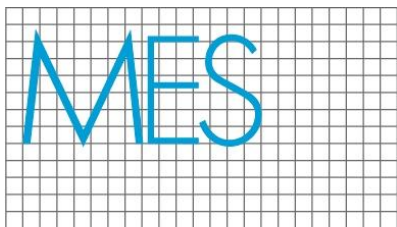
SPONSORS





HEMEDEX[®]





FORSCHUNGSSYSTEME GMBH



30  **moor instruments**
years of microvascular assessment



 **RAUMEDIC®**
— *Lifeline to Health* —

Where to find us.....

Our venue is located on Charité Campus Mitte – very much in the center of Berlin!

It is conveniently accessible by various means of public transportation.

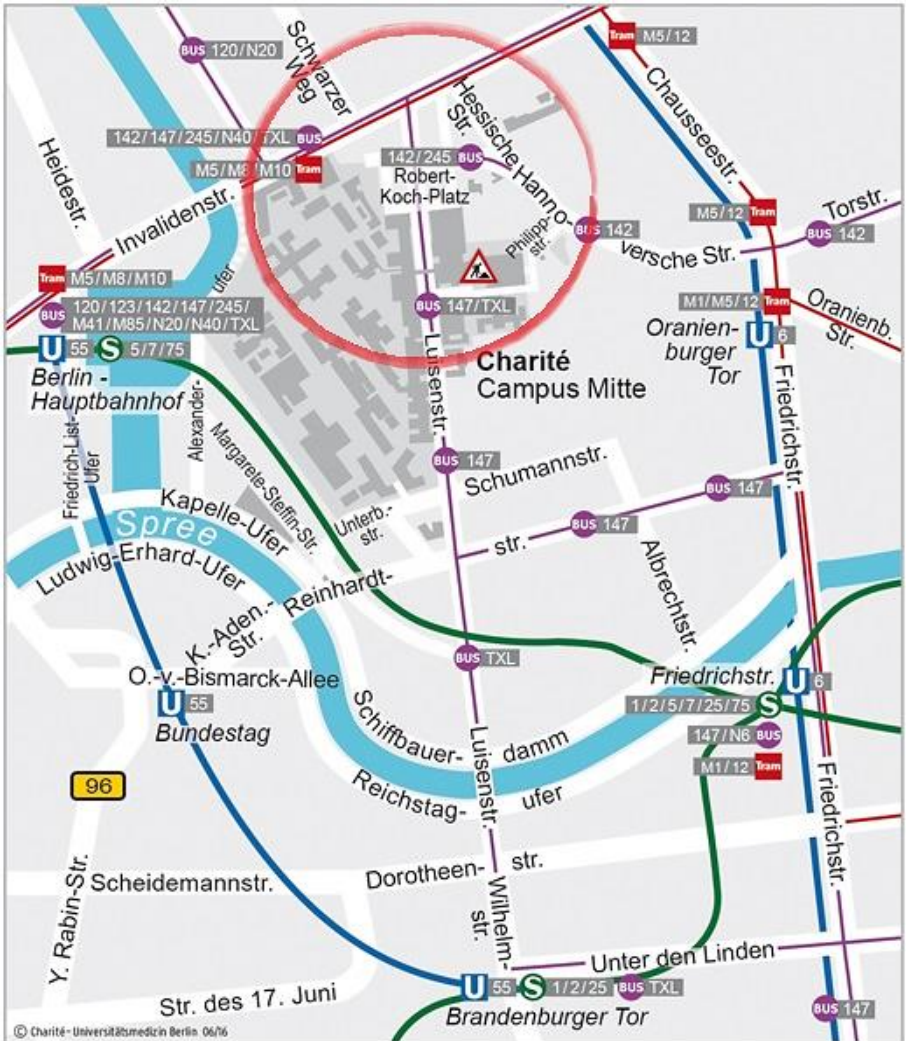
Closest stops – within walking distance to Charité – are

Berlin Hauptbahnhof:	S Bahn (city train lines) S5, S7, S75 U Bahn (subway line) U55 Tram M5, M8, M10 Bus 120, 123, 142, 147, 245, EV, M41, M85, TXL (to Tegel airport) various regional, national and international trains
U Naturkundemuseum:	U Bahn U6 Tram M5, M8, M10, 12
Invalidenpark:	Tram M5, M8, M10 Bus 120, 142, 147, 245, TXL

Address

Kaiserin-Friedrich-Haus
Robert-Koch-Platz 7
10115 Berlin





The dinner venue is at the Hörsaalruine also on Charité Campus Mitte, about 5 minutes walking from the Kaiserin-Friedrich-Haus where the meeting will be held.

