EPITARGET

EPITARGET – Young Researchers' Symposium

Marseille – 26 October 2016

PROGRAMME & ABSTRACT BOOKLET

EPITARGET – Young Researchers' Symposium

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EPITARGET Poster Session

Abstracts

Plasma miR-124 as a potential biomarker for early diagnosis of epileptogenic brain damage after TBI

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Traumatic brain injury (TBI) causes about 5% of all and 20% of acquired epilepsy. Posttraumatic epileptogenesis occurs in parallel to the progression of secondary brain damage, even days or months after TBI. MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate protein synthesis at the post transcriptional level. Alterations in miRNA levels are predicted to occur earlier in response to a disease state than conventional protein biomarkers, thus making them attractive for detecting early stages of disease development, including epileptogenesis.

In order to assess the potential of miRNAs as plasma biomarkers for posttraumatic epileptogenesis, we identified miRNAs which regulated brain transcriptomic changes at 3 months after lateral fluid-percussion-induced (FPI) TBI, that is, when rats are undergoing epileptogenesis. Bioinformatics analysis using Ingenuity Pathway Analysis (IPA®) suggested brain-specific miR-124 as one of the regulators of chronic transcriptomic upregulation. Next we characterized the levels of miR-124 in the blood/plasma at different time points after TBI.

Overall, in the controls, the level of miR-124 in the blood was very low, being close to detection limit when assessed using quantitative reverse-transcription PCR (qRT-PCR). The blood level of miR-124 was elevated at 2 d after lateral FPI (FC=4.63, $p<0.01$) returning back to normal by 2-months post-injury. Further analysis of miR-124 in plasma, sampled at 2d post-TBI, using both droplet digital PCR (ddPCR) and qRT-PCR confirmed the elevation in miR-124 levels (ddPCR: FC=1.37, $p<0.05$; qRT-PCR: FC=1.8, $p<0.05$). Further, there was a good correlation between these two methods (ρ =0.74, $p<0.01$). Receiver Operating Characteristic (ROC) curve indicated that plasma miR-124 distinguished the post-TBI rats from the sham-operated controls with an AUC of 0.817 for ddPCR ($p<0.05$)

and 0.833 for qRT-PCR ($p<0.05$). Finally, we asked if there is an association of plasma miR-124 with the evolution of brain damage. Based on cortical lesion endophenotype, rats were grouped into chronic inflammatory (TBI_{CI}) or cavity-forming (TBI_{CF}) endophenotypes. Interestingly, rats destined to develop the TBI_{CF} endophenotype over the 3-month follow-up had an elevated plasma miR-124 level at 2 d post-TBI. ROC analysis revealed an AUC of 0.917 ($p<0.05$) for ddPCR and 1.00 ($p<0.05$) for qRT-PCR when comparing the TBI_{CF} group to sham-operated controls. When qRT-PCR results were analysed, ROC also differentiated the TBI_{CI} and TBI_{CF} lesion endophenotypes (AUC=0.917, $p<0.05$).

Our analysis indicated that elevated brain-specific miR-124 could be a good plasma biomarker candidate for TBI and consequent epileptogenesis as its concentration is low in normal animals. Further, our preliminary data suggest that increased plasma miR-124 signals about the evolution of cortical lesion endophenotype as rats with TBI_{CF} endophenotype tended to have a higher concentration of miR-124 as compared to TBI_{CI} endophenotype. Further analysis will reveal the potential of miR-124 as a biomarker for posttraumatic epileptogenesis.

The intrahippocampal kainate model in mice - Effects of strain, sex, anesthesia, and EEG seizure definition

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The intrahippocampal mouse model of acquired epilepsy represents a valuable model of mesial temporal lobe epilepsy with hippocampal sclerosis. After intrahippocampal injection of kainate mice develop convulsive seizures as well as highly frequent electrographic seizures that are resistant to common antiseizure drugs, including carbamazepine (CBZ).

The goal of this study was to compare the efficacy of CBZ in this model in two different mouse strains (FVB/N and NMRI) and to assess the influence of the definition of electrographic seizures on the antiseizure efficacy.

We evaluated the effect of CBZ separately for the two occurring types of electrographic seizures: High voltage sharp waves (HVSWs) and hippocampal paroxysmal discharges (HPDs). In addition, we used different spike frequencies, interevent intervals, and amplitudes for HVSW-definition and exemplarily assessed the antiseizure efficacy of diazepam and phenobarbital.

Female FVB/N mice developed frequent HVSWs but only rarely HPDs and secondarily generalized convulsive seizures. Only slight changes in inclusion and exclusion criteria of HVSWs determined whether they were resistant or responsive to CBZ. Male NMRI mice exhibited both types of electrographic seizures, HVSWs and HPDs., HVSWs were more resistant than HPDs to treatment with CBZ. Diazepam and Phenobarbital suppressed both types of electrographic seizures.

The data demonstrate that genetic background as well as seizure definition critically determined whether electrographic seizures are responsive or resistant to CBZ.

Circulating microRNA as a biomarker of epileptogenesis and epilepsy in the rat model of temporal lobe epilepsy

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Epilepsy frequently develops as a result of brain insult, ex. brain injury, stroke, inflammation or status epilepticus, however currently there are no tools allowing us to predict which patients suffering from trauma will eventually develop epilepsy or how severe it is going to be. In recent years small non-coding RNAs are proposed as biomarkers for neurological diseases. Particularly microRNAs are interesting candidates, as several of them were described as changing its levels in the brain of epileptic patients and in epilepsy animal models. There is evidence suggesting that microRNAs levels are altered also in the plasma of epileptic subjects, making them attractive candidates for peripheral biomarkers of epilepsy. This study was conducted to evaluate usefulness of plasma miRNAs as biomarkers of epileptogenesis and epilepsy.

In our studies we used the rat model of temporal lobe epilepsy. The status epilepticus was evoked by 25 min stimulation of the left lateral nucleus of amygdala (100-ms train of 1-ms biphasic square-wave pulses; 400 µA peak to peak, delivered at 60 Hz every 0.5). Animals were continuously video and EEG monitored for 6 months to detect spontaneous seizures. Blood was collected at 14, 30, 60, and 90 days after stimulation from tail vain. Blood plasma was separated and processed using Affymetrix miRNA 4.1 array strip microarrays.

We have compared miRNA levels between sham operated (n=12) and stimulated animals (n=15); $p<0.01$ was used as a cut off. We have detected 14 miRNA differentiating between sham operated and stimulated animals at 14 days, 6 at 30 d. We have also compared the miRNAs levels between animals with high (30-70 seizures/day) and low (1-5 seizures/day) number of seizures. We have found differences in levels of 11 miRNA at 14 d, 7 at 30 d (at $p<0.01$).

Levels of miRNA in plasma are altered following epileptogenic stimulus and differentiate between animals with frequent and rare seizures. miRNA may become a useful peripheral biomarker of epileptogenesis/epilepsy as well as severity of the disease.

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Meta-analysis of miRNA profiles in rodent epilepsy models; challenges, perspective and relevance to humans

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Over the last decade various high-throughput studies using microarrays and RT-qPCR arrays have demonstrated the differential expression of numerous miRNAs in the brain tissue of different rodent models of epilepsy. While these studies have produced vast amounts of data and identified numerous potential miRNA targets, the results are largely discordant, varying greatly from model-to-model and even from lab-to-lab. Here, using the robust rank aggregation method we performed a comprehensive meta-analysis across 23 different studies from rodent post-status epilepticus (SE) models, including electrical-stimulation, kainic acid and pilocarpine models during the acute, latent and chronic phases. This led to the identification of miR-132-3p, miR-21-5p, miR-21-3p and miR-212-3p at the acute stage, miR-21-5p, miR-132-3p, miR-212-3p, miR-142-5p, miR-139-5p and miR-33-5p at the latent stage and miR-23a-3p, miR-135b-5p and miR146a-5p at the chronic stage as consistently differentially expressed across the surveyed studies. Pathway analysis revealed that while the acute and latent stages of epilepsy are characterised by disturbances to the MAPK signalling pathway and cytokine-cytokine receptor interaction, the chronic stage involves disturbances to the NF-κB signalling pathway. Overall the identified miRNAs provide interesting targets for further investigation into the molecular pathology of epilepsy. Furthermore, we also discuss the challenges faced during this study, relevance of these results to human epilepsy, and prospective future miRNA expression studies using small RNA-Sequencing.

Seizure-induced microvascular pathology: the role of glutamate

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Brain microvasculature is unique and includes the blood-brain barrier (BBB) that controls molecular transfer to maintain neuronal homeostasis. Microvascular dysfunction, often associated with increased permeability of the BBB, features common systemic diseases with neurological manifestations as well as primary brain disorders including epilepsy. Epilepsy is characterized by seizures – episodes of hypersynchronized neuronal activity that is associated with excessive release of glutamate. Since seizures are often associated with microvascular dysfunction, we tested the hypothesis that glutamate directly increases endothelial permeability. Using intravital microscopy and the cranial open window approach, we induced recurrent seizures in anesthetized rats by topical application of 4-aminopyridine on the surface of the neocortex. We demonstrate that a single seizure is associated with increased BBB permeability. Topical application of glutamate in the presence of blockers of neuronal activity and synaptic transmission, increased vascular permeability as well. This effect was replicated with agonists to N-Methyl-D-aspartate (NMDA) receptors and prevented with APV, an NMDA receptor antagoni1st. We further show that glutamate induces calcium influx and increased nitric oxide in close proximity or within microvasculature endothelium. These new findings suggest that NMDA antagonists may have a protective role by directly targeting cerebral vasculature, but also offer a new approach to enhance drug delivery by neuronal activation. We exemplify that NMDA receptor antagonists diminish BBB dysfunction following recurrent seizures. Furthermore, we demonstrate that low frequency transcranial magnetic stimulation (TMS) enhances vessel permeability in a manner allowing increased drug delivery in anesthetized rodents. We followed these pre-clinical trials with a clinical pilot study in which we demonstrated enhanced brain vessel permeability following deep TMS, in grade IV glioblastoma patients.

Seizures induce microvascular injury involving pericytic dysfunction, neurovascular decoupling and a leaky blood-brain barrier

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The burden of comorbidities in epilepsy is high, and brain disorders such as depression, anxiety and dementia are more common in people with epilepsy than in the general population. While several studies have suggested either shared risk-factors or bidirectional relations between epilepsy and neurological comorbidities, the mechanisms underlying this association are yet to be understood. We have previously shown that long-lasting dysfunction of the blood-brain barrier (BBB) leads to astrocytic transformation, a neuroinflammatory response, excitatory synaptogenesis and pathological plasticity. Inversely, vascular injury and specifically BBB dysfunction (BBBD) are well-documented outcomes of seizures, and have been shown in animal models and in resected tissue of epileptic patients. However, the mechanisms that underlie BBBD are poorly understood, and while different processes were suggested to contribute to cellular damage, the interplay/causality between these events and BBBD remains to be clarified.

The elements of the neurovascular unit and proper interactions between them underlie the regulation of oxygenated-blood supply to activated cells. In this homeostatic mechanism, termed functional hyperemia or neurovascular coupling (NVC), blood vessels dilate to match the metabolic demands of neurons and astrocytes. However, whether this coupling is preserved despite the extremely high metabolic demands of seizures is still a matter of debate and increase the importance of understanding the potential role of neurovascular decoupling (NVD) in seizure disorders.

As pericytes are key executors of NVC in capillaries, play a crucial role in BBB integrity, and were shown to redistribute following seizures, their functionality during and following

seizures may be of particular relevance. Using the organotypic slice culture preparation we demonstrate trans-membrane pericytic inward currents during seizures, free radicals (ROS) formation, impaired pericytic motility and microvascular dysfunction during recurrent seizures. We further performed in vivo experiments in anaesthetized rats confirming the in vitro observations and demonstrating cellular injury, BBBD and NVD during recurrent seizures induced by topical neocortical application of the K^+ channel blocker 4-amino-pyridine. Despite NVC impairment, late seizures were not accompanied by reduction of tissue oxygenation or altered potassium homeostasis, thus ruling out hypoxia as a mediator of enhanced cellular damage and potassium to underlie NVD. Interestingly, the ROS-scavenger Tempo, while reducing cellular injury, did not affect BBBD, nor rescued the NVD. Our results point to ROS-independent pericytic dysfunction as a key underlying mechanism contributing seizure-induced microvascular injury.

Tracer Development for MAO-B Imaging of Epileptogenesis – An Update

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Brain inflammation is a pathological hallmark of epilepsy, and results from several biological pathways activated following brain injury. Imaging of these pathways in longitudinal studies is of particular interest as it may reveal valuable information about both the chronology, the underlying mechanisms of epileptogenesis as well as surrogate imaging biomarkers for treatment response. Amongst the different targets involved in neuroinflammation, Monoamine Oxidase-B (MAO-B) is a biomarker that reflects astroglial activation.

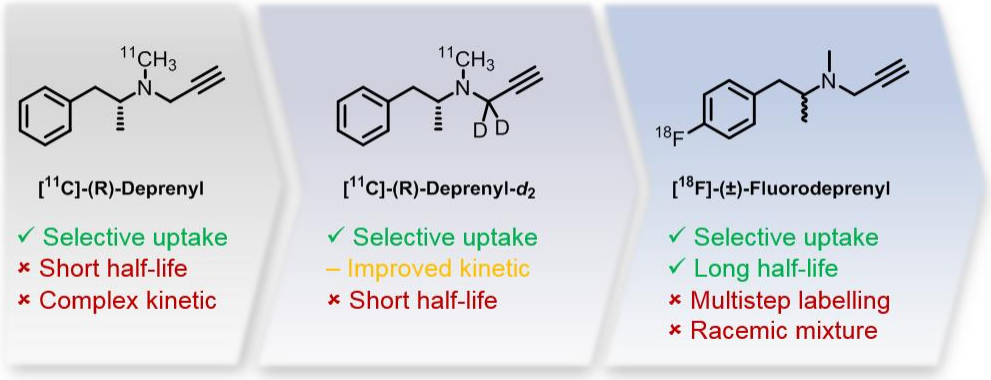

Figure 1 Different generation of deprenyl-based MAO-B tracers

Imaging of MAO-B in epileptic patients using ^{11}C-L-deprenyl has been reported in the late 80's (Figure 1).[1] In spite of promising results, the practical use of this tracer has been hampered by the short half-life of carbon-11 (20 min) and by the difficult deconvolution of the data arising from the specific kinetic of the tracer.[2] Since these initial results, several novel deprenyl-based MAO-B tracers have been developed, but none of them addressed all the issues of ^{11}C-L-deprenyl (Figure 1).[3]

In an attempt to develop an ideal radiotracer for imaging MAO-B, we are focusing our efforts on the synthesis of fluorinated version of deprenyl. The design of this tracer, as well as the current development status will be discussed.

Kinetic analysis improves evaluation of microglia activation in epileptogenesis.

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Evidence indicates that, after an insult, brain inflammation may contribute to epileptogenesis. The aim of this study was (1) to characterize the timeline of microglia activation after pilocarpine-induced status epilepticus (SE), and (2) to evaluate the effect of anti-inflammatory treatment with minocycline, using TSPO PET.

[1] Fowler J. S. et al., *Science*, **1985**, *235*, 481–485
[2] Bergström M. et al., *Acta Neurol Scand*, **1998**, *98*, 226–231
[3] a) Plenevaux A. et al., J. *Med. Chem*., **1990**, *33*, 2015–2019; b) Fowler J. S. et al., *J. Nucl. Med*., **1995**, *36*, 1255–1262; c) Nag S. et al., *J. Med. Chem*., **2011**, **54**, 7023–7029; d) Nag S. et al., *J. Nucl. Med*., **2016**, *57*, 315–320.

Epileptogenesis was initiated by pilocarpine-induced SE. The temporal profile of neuroinflammation was assessed in rats at 8 time points (n=4-8) over 16 weeks after SE performing ^{11}C-PK11195 PET imaging. Subsequently, additional rats were treated with either minocycline (25 mg/kg) or vehicle for 8 days starting 24 hours after SE and underwent TSPO-PET scans before SE (n=9), and at 7 and 14 days post SE (n=5-7 per time point and treatment group). Sixty-minute dynamic scans were performed in rats anesthetized with isoflurane for both tracers. Images were analyzed in Pmod software by comparing standardized uptake values (SUV) and binding potential (BP_{ND}; simplified reference tissue model) using cerebellum as reference region. In addition, statistical parametric mapping (SPM12) was used for comparing each time point to baseline. For treatment evaluation, volume of distribution (V_t; 2-tissue compartment model) was also calculated as the dynamic images allowed for determination of an image-derived input function.

Compared to baseline, ^{11}C-PK11195 uptake values were increased in epileptogenesis-associated brain regions at 48 hours after SE and peaked at 7 days after SE (up to 2.09-fold increase in uptake, $p < 0.05$). Interestingly, BP_{ND} peaked later than uptake values, with maximal BP_{ND} observed at 14 days in ventral hippocampus (0.96±0.18; $p<0.05$). Uptake and BP_{ND} remained above baseline values up to 3 weeks after SE. During anti-inflammatory treatment evaluation, elevated TSPO-tracer uptake, BP_{ND} and V_t were observed at both investigated time points after SE in both groups. Minocycline treatment decreased the V_t in the ventral hippocampus (3.72 ± 0.25 vs. 2.66 ± 0.53, $p < 0.05$) and entorhinal cortex (3.66 ± 0.32 vs. 2.77 ± 0.38, $p < 0.05$) at 1 week post SE, and in the piriform cortex at both time points compared to the vehicle-treated group (1 week: 3.58 ± 0.30 vs. 2.83 ± 0.33, $p < 0.05$; 2 weeks: 3.29 ± 0.04 vs. 2.54 ± 0.07, $p < 0.05$). No difference was found in SUV or BP_{nd}.

In-depth kinetic analysis of PET data allows for exact determination of neuroinflammation time profile after epileptogenic brain insult, which enables appropriate timing of inflammation-targeting antiepileptogenic pharmacotherapy. Furthermore, it allows evaluation of anti-inflammatory treatment effects.

Somatostatin immunoreactive interneurons survival in the intrahippocampal kainate model

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Epilepsy is a neurological disorder affecting 1% of the world population. Despite decades of effort for drug development, still 30% of the patients are pharmacoresistant. Novel strategies to treat the hyper excitability in the pharmacoresitant affected networks, usually located in hippocampus, are needed. Recent evidence shows that optogenetic activation of inhibitory cells in hippocampus interferes with seizure generation[4] in naïve animals. To predict whether such approach could also be effective in an epileptic hippocampus, where inhibitory neurons may degenerate, we characterized survival of somatostatin-expressing interneurons (SST) in the unilateral intrahippocampal kainic acid (KA) rat-model of chronic TLE. The results show a survival of SST interneurons in the contralateral side to the kainite injection, along with an increase in their soma diameter. These data indicate that the SST cells that survive in the contralateral site to the kainite injection pose a promising target for optogenetic approach to understand icotgenesis in epileptic tissue, and for possibly treating refractory epilepsy.

Collagen VI extracellular matrix protein modulates short-term plasticity in the hippocampus: Implications for epileptogenesis.

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[4] Ledri, M., Madsen, M. G., Nikitidou, L., Kirik, D. & Kokaia, M. Global optogenetic activation of inhibitory interneurons during epileptiform activity. *J Neurosci* **34**, 3364-3377, doi:10.1523/jneurosci.2734-13.2014 (2014).

Extracellular matrix (**ECM**) molecules modulate several aspects of synaptic plasticity in the central nervous system (**CNS**) through multiple mechanisms that ultimately affect learning and memory. Collagen VI (**CVI**) belongs to the category of ECM protein and is primarily known for its bridging role in connective tissues. Here we report that the mRNA and protein levels of Collagen VI (**CVI**) are increased in the rat hippocampus at 4 weeks after the initial insult in post status epilepticus model of chronic epilepsy. In addition, we further explore whether CVI plays a role in synaptic plasticity and if it can functionally affect neuronal networks in the hippocampus. Surprisingly, we detect an increased basal synaptic transmission after exposing rat hippocampal slices to CVI for 2 h and paired-pulse facilitation in Schaffer collateral-CA1 excitatory synapses after incubating hippocampal slices with CVI for 6 h, whilst CVI deficient mice exhibited paired-pulse depression, the opposite effect. These data suggest that CVI may decrease overall release probability in Schaffer collateral-CA1 synapses and, since CVI protein levels are augmented in post-status epilepticus animals, this could help in counteracting increased excitability in epileptic neuronal circuits.

Molecular isoforms of High Mobility Group Box 1 in blood are novel mechanistic biomarkers for epileptogenesis

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The neuroinflammatory High Mobility Group Box-1/Toll-like Receptor 4 (HMGB1/TLR4) axis is activated in brain epileptic foci and contributes to experimental seizures generation. Posttranslational modifications of HMGB1, such as acetylation and oxidation/reduction, determine its interactions with the receptors and modulate its biological activity. We found out that among the different isoforms, disulfide HMGB1 promotes seizures generation and recurrence and enhances glutamate excitotoxicity. Based on evidence that HMGB1 is a soluble neuroinflammatory molecule that contributes to seizure mechanisms, and it can be measured stably in blood, we determined if total HMGB1 and its isoforms can be used as non-invasive, mechanistic biomarkers of

neuroinflammation and epileptogenesis and tested if they predict antiinfammatory treatment response.

We used two rat models of epileptogenesis evoked by SE. In the first one, adult male rats were electrically stimulated in the ventral hippocampus to induce SE, and all rats developed epilepsy. Then, rats were randomized in two arms: one group of rats was treated with a combination of anti-inflammatory drugs for 7 days before disease onset (i.e~ 14 days after SE) and one group received the corresponding vehicles. The drug combination was rationally designed to effectively block the activation of the IL-1R1/TLR4 inflammatory pathway. To study the effect of treatment on epileptogenesis, rats were EEG recorded for 2 weeks rat 2 and 4 months after SE to determine the progression in seizure frequency during the disease development. In the second model, SE was induced in post-natal day 21 (PN21) male rats with intraperitoneal injection of lithium+pilocarpine. In this model, 60-70% of rats develop spontaneous seizures in the adulthood and onset of disease is around 70-90 days post-SE. Using mass spectrometry analysis, we measured total HMGB1, and its acetylated, reduced and disulfide isoforms before epilepsy onset and during disease development in the two models of symptomatic epilepsy, correlating brain changes to those in blood.

In adult rats exposed to electrical SE, acetylated and disulfide HMGB1 are up-regulated following SE and brain changes of these isoforms precede the respective blood increase. Moreover, HMGB1 was upregulated before epilepsy onset and progressively increased during chronic epilepsy. In PN21 rats exposed to SE, blood levels of HMGB1 measured before the expected time of epilepsy onset predicted which rats will develop the disease. In the electrical model, the anti-inflammatory treatment prevented the progression in seizure frequency observed in vehicle rats between 2 and 4 months post-SE. Total HMGB1 predicted the therapeutic response to anti-inflammatory drugs when measured before epilepsy onset.

We identified dynamic changes in HMGB1 isoforms in the brain and blood of rats undergoing epileptogenesis following SE. Notably, HMGB1 isoforms increased in blood before disease onset and prospectively identified animals developing epilepsy. Treatment of rats with anti-inflammatory drugs during epileptogenesis arrested disease progression and prevented the blood increase in HMGB1 isoforms. Circulating HMGB1 may serve as a mechanistic biomarker of neuroinflammation and epileptogenesis and for predicting the therapeutic response to anti-inflammatory drugs.

Further studies examining the utility of HMGB1 isoforms as biomarkers in patients exposed to a potential epileptogenic injury or with a first presentation of seizures are in progress.

Effects of strain, sex, anesthesia, and EEG seizure definition on the intrahippocampal kainate model in mice

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The intrahippocampal kainate model of mesial temporal lobe epilepsy is frequently being used for studies on epileptogenesis and antiepileptogenesis. Notwithstanding the fact that gender may affect susceptibility to epilepsy, only male mice were used in almost all previous studies.

The main goal of this study was to investigate the influence of sex on epileptogenesis in the intrahippocampal kainate mouse model. Furthermore we also examined the influence of different mouse strains, type of anesthesia during SE (chloral hydrate vs. isoflurane) and the injected dose of kainate on the development of status epilepticus (SE), epileptogenesis and chronic epilepsy.

Therefore continuous (24/7) video-EEG monitoring was performed during SE and the following 2 weeks, as well as 4-6 weeks after SE.

In agreement with previous studies, male NMRI mice with chloral hydrate anesthesia showed a seizure-free latent period of 10-14 days and frequent hippocampal paroxysmal discharges (HPDs) in the chronic phase. When using isoflurane anesthesia, SE was more severe and not all male mice exhibited a seizure-free latent period.

In apparent contrast, female NMRI mice did not show any clear seizure-free latent period after SE, independently of anaesthesia type, and HPDs were only rarely observed. Neither decreasing the dose of kainate, nor using female mice from different mouse strains (C57BL/6N and FVB/N) led to a clear latent period and higher frequencies of HPDs.

This is the first study to demonstrate a marked sexual dimorphism in epileptogenesis in a rodent model of acquired epilepsy and will most certainly affect the preclinical antiepileptogenesis study design. In addition to that, our data show that the type of anaesthesia not only affects SE severity, but also the characteristics of the following latent period.

Encephalitis-induced epilepsies: Studies with the Theiler's murine encephalomyelitis virus (TMEV)

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Central nervous system infections are among the main causes of acquired epilepsies. The availability of animal models of viral infection-induced epilepsy is very limited. One promising model is the infection with Theiler's murine encephalomyelitis virus (TMEV). After intracerebral infection, about 50% of C57BL/6 (B6) mice develop acute (early) as well as chronic epileptic seizures, whereas SJL mice develop a demyelinating disease without seizures. This prompted us to investigate the prerequisites for epilepsy development after TMEV infection. We hypothesized that apart from mouse strain differences, also virus substrain differences as well as infiltrating immune cells might be responsible for seizure development.

We infected different substrains of SJL and B6 mice intracerebrally with three different TMEV strains: BeAn-1, BeAn-2, and DA. Investigated parameters were: clinical status, early and late seizure occurrence, hippocampal immune cell infiltration, expression of the interferon-inducible antiviral effector ISG15, and hippocampal damage.

B6 mice developed seizures to a varying degree depending on the virus strain: Infection with BeAn-2 and DA induced early and late seizures in B6 mice. Contrary, BeAn-1 infection was not able to induce any seizures, but caused a more severe demyelinating disease in SJL mice and increased T-cell infiltration in B6 mice. Receiver operating characteristic curve analysis revealed that neurodegeneration, ISG15 expression, and microglia/macrophage activation predicted the occurrence of early seizures.

Overall, the TMEV model is more complex than previously thought and strongly depends on the virus and mouse substrain used, thus providing a unique platform to study virus and host factors in ictogenesis and epileptogenesis.

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Identification of a transcription factor controlled neuronal transcript signature activated early after status epilepticus

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Temporal lobe epilepsy (TLE) is generally characterized by recurrent spontaneous seizures that emerge from a hyperexcitable hippocampal formation. In many patients, TLE develops after transient insults to the brain including status epilepticus (SE). However, the mechanisms that convert a normal into a chronically hyperexcitable hippocampus, often referred to as epileptogenesis, are only incompletely understood. Here, our goal was to characterize transcription factor controlled expression signatures in early epileptogenesis putatively promoting the epileptogenic process.

To this end we induced SE by systemic application of pilocarpine to mice and sacrificed them at 2h, 6h, 12h, 24h and 36h after SE. Next we micro-dissected the CA1 region and isolated mRNA for Next Generation Sequencing.

In order to identify relevant signalling cascades during *early* epileptogenesis we first performed bioinformatic analysis of this mRNA-Sequencing data set. Using BioLayout3D, genes were clustered in co-expression networks revealing genes that exhibited similar changes in expression at different time points after SE. To identify those genes in the clusters with substantial expression in neurons we filtered our data set against a published database of gene expression levels in glia, neurons, and vascular cells of the cerebral cortex using a homemade perl script. We identified 11015 genes that were clustered in a neuronal co-expression network and five clusters showed an up-regulation

of gene expression at distinct time points after SE. We then searched for transcription factor (TF) binding sites that are present in the promoters of most of the clustered genes using Cytoscape. Also in this analysis TFs were filtered for significant neuronal expression. We then focused on one expression cluster, which shows a striking up-regulation already two hours after SE and consists of 170 genes. A gene ontology analysis revealed that this cluster contains 25 TFs. The Cytoscape prediction showed that the promoters of these 25 TFs are enriched in potential TF binding sites for six TFs. Of these, three have already been linked to epilepsy (Egr1, Jun, and NPAS4), whereas the other three, Sox11, Zbtb14 and Fezf2, have so far not been studied in this context. Functional verification experiments are currently ongoing and respective data will be presented.

In conclusion, our data suggest distinct gene expression signatures controlled by particular sets of TFs related to key functional aspects of epileptogenesis including Jun and Egr1. Transient antagonism of respective TFs after potentially epileptogenic brain insults may be effective in blocking epileptogenic gene expression dynamics and attenuate or retard spontaneous seizure emergence and thereby provide new therapy perspectives.

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Controlling Epileptiform Activity with Implantable Organic Electronic Ion Pumps

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In treating epilepsy, the ideal solution is to act at a seizure's onset, but only in the affected regions of the brain. Here, an implantable organic electronic ion pump (OEIP) is demonstrated, which directly delivers on-demand pure molecules to specific brain regions. State-of-the-art organic devices and classical pharmacology are combined to control pathological activity in vitro, and the results are verified with electrophysiological recordings. We use three different models to induce epileptiform activity in vitro and show that delivery of gamma-aminobutyric acid (GABA) results in quick and localized suppression of this activity. As the integration of OEIPs on implantable probes is rather

straightforward, we believe that these devices have great potential in drug delivery in the brain, and in particular in delivery of antiepileptic active substances.

Longitudinal TSPO-PET imaging of brain inflammation in the intrahippocampal kainate mouse model

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Accumulating evidence suggests that brain inflammation, elicited by epileptogenic insults, may be involved in epilepsy development. Here we investigated upregulation of the translocator protein (TSPO) by serial PET imaging in the intrahippocampal (ihc) kainate mouse model.

Status epilepticus (SE) was induced in mice by ihc injection of kainate. Mice underwent TSPO-PET scans before SE, at 2 days, 5-7 days, 2 weeks, 3 weeks, and 14-15 weeks post SE. Sham-injected mice were scanned in parallel. For data evaluation, percentage injected dose/cc (%ID/cc) was calculated using a VOI template atlas.

In excess of evident focal microglia activation in the injection site of sham mice, following SE, elevated uptake was observed in the ipsilateral hippocampus and to a lesser extent in the contralateral hippocampus, from 2 days until at least 7 weeks, with a peak at 5-7 days post SE. Moderately enhanced tracer uptake was also evident in the ipsilateral thalamus at 2 days post SE.

Compared to microglia activation in the pilocarpine rat model, here, inflammatory processes mainly affected the hippocampus ipsilaterally to the injection site but are also apparent in the contralateral hippocampus, and remained distinctly present in the chronic phase of epilepsy.

2-deoxy-D-glucose-mediated deceleration of kindling-induced epileptogenesis is reflected by ^{18}F-FDG brain kinetics

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A therapeutic option to hold epileptogenic processes leading to acquired epilepsy is still lacking. Recent studies suggest that modulation of cerebral glucose metabolism by 2-deoxy-D-glucose (2-DG) might exert anti-epileptogenic effects. Here, we applied ^{18}F-FDG PET to investigate effects of 2-DG treatment during epileptogenesis.

6Hz-corneal kindling was performed in mice by twice daily electrical corneal stimulation for 21 days. Seizure response was scored using a modified Racine scale. Saline (n = 12) or 250 mg/kg 2-DG (n = 18) were injected i.p. 18 h before baseline ^{18}F-FDG PET scans and subsequently 1 min after each stimulation. Twelve additional mice received 2-DG without kindling. Dynamic 60-min ^{18}F-FDG PET/CT scans were acquired at baseline and on days 10 and 17. A standard MRI-based brain atlas was used to quantify ^{18}F-FDG uptake. Kinetic modelling was performed to evaluate glucose metabolic rate MRGlu and uptake rate constant K_i. Expression of glucose transporter 1 (GLUT-1) and activation of astrocytes (GFAP) and microglia (IBA1) was immunohistochemically analysed.

Kindling progression was attenuated by 2-DG-treatment, mainly in the early phase (up to 29.3 ± 11.8%, p=0.0009). Kindling in combination with 2-DG treatment increased ^{18}F-FDG uptake by up to 36.0 ± 10.3% (p=0.0016) at day 10 in hippocampus, compared to the unkindled 2-DG treated group. At day 10, the 2-DG treated kindling group showed an up to 1.32 fold increase in influx constant K_i compared to the unkindled group as well as a higher MR_{Glu} at day 17 than the saline-treated kindling group. In saline-treated mice, kindling did neither alter ^{18}F-FDG uptake nor kinetic parameters, but induced astrocytic activation. GLUT-1 expression remained unaltered by kindling or 2-DG treatment, whereas microglia was strongly activated in 2-DG-treated animals, irrespective of animals being kindled or not.

2-DG-treatment during kindling-induced epileptogenesis is accompanied by persistent increases in ^{18}F-FDG brain turnover. Interestingly, repetitive 2-DG application also leads to significant microglia activation.

^{18}F-FET PET during epileptogenesis reveals amino acid transport timeline

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^{18}F-FET PET allows to measure amino acid transport, indicative of metabolically active cells, and its distribution within the brain. This study aimed to longitudinally characterize brain amino acid transport during epileptogenesis.

Status epilepticus (SE) was induced by lithium-pilocarpine in female Sprague-Dawley rats. ^{18}F-FET (19.26±0.26 MBq) was injected via a catheter inserted into a lateral tail vein at the same time that a dynamic 60-min PET acquisition was started. ^{18}F-FET images were analysed by co-registering them to a template MRI and applying a VOIs atlas using Pmod software. Tracer uptake was calculated as uptake ratio (SUVR) to pons as reference region, and volume of distribution (V_t) was obtained by applying Ichise's multilinear analysis, a further development of the Logan Plot aimed at minimizing the bias induced by noise in the measurements.

Kinetic analysis showed a decrease of amino acid transport at 48 h post SE in amygdala and hippocampus, recovering baseline levels at 7-14 days. In the chronic state (10-14 weeks), both, V_t and SUVR, showed a reduction in the ^{18}F-FET uptake in the hippocampus.

This study suggests that amino acid transport is altered during early epileptogenesis and in chronic epilepsy. While the early reduction of ^{18}F-FET uptake might be indicative for insult mediated acute cell damage, the reduction in the chronic phase of this model is probably due to regional atrophy.

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