EPITARGET - Young Researchers' Symposium & Poster Session

Istituto di Ricerche Farmacologiche Mario Negri

Programme & Abstract Booklet

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EPITARGET Young Researchers' Symposium

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Abstracts

Magnetic resonance imaging-based quantification of blood-brain barrier permeability: a novel biomarker in epileptogenesis?

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Accumulating clinical and animal data strongly indicate the involvement of blood-brain barrier dysfunction in fostering brain inflammatory response and epileptogenesis. Furthermore, pharmacological interventions in experimental animal suggest that targeting blood-brain barrier pathology and associated inflammatory signaling may be promising new tools in preventing epilepsy following brain injury and associated degenerative changes. These experimental data call for the development of reliable diagnostic methods that will measure vascular integrity as a biomarker for epileptogenic changes in the local network. These methods are expected to allow the reliable identification of blood-brain barrier dysfunction and local brain inflammatory response, localize the epileptogenic zone and identify patients at-risk for epilepsy. The development of a reliable biomarker will allow the selection of patients for targeted treatment(s), assessing patients' response to treatment and help in rationale decision for the required dose and duration of treatment. We have thus developed several approaches to reliably and quantitatively detect blood-brain barrier dysfunction using contrast-enhanced magnetic resonance imaging (CE-MRI). The goals of this presentation are to: (1) demonstrate CE-MRI-based imaging and analysis methods allowing a quantitative measure for vascular permeability; (2) demonstrate the implementation of these methods and their use in animal models of epileptogenesis, including status epilepticus and brain injury; (3) demonstrate feasibility in human studies; and (4) discuss the potential and limitations of blood-brain barrier imaging as a future biomarker of cortical tissue at-risk for epilepsy and neurodegeneration.

MicroRNA profiles in hippocampal granule cells and plasma of rats with pilocarpine-induced epilepsy – comparison with human epileptic samples.

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The identification of biomarkers of the transformation of normal to epileptic tissue would help to stratify patients at risk of epilepsy following brain injury, and inform new treatment strategies. MicroRNAs (miRNAs) are an attractive option in this direction. In this study, miRNA microarrays were performed on laser-microdissected hippocampal granule cell layer (GCL) and on plasma, at different time points in the development of pilocarpine-induced epilepsy in the rat: latency, first spontaneous seizure and chronic epileptic phase. Sixty-three miRNAs were differentially expressed in the GCL when considering all time points. Three main clusters were identified that separated the control and chronic phase groups from the latency group and from the first spontaneous seizure group. MiRNAs from rats in the chronic phase were compared to those obtained from the laser-microdissected GCL of epileptic patients, identifying several miRNAs (miR-21-5p, miR-23a-5p, miR-146a-5p and miR-181c-5p) that were up-regulated in both human and rat epileptic tissue. Analysis of plasma samples revealed different levels between control and pilocarpine-treated animals for 27 miRNAs. Two main clusters were identified that segregated controls from all other groups. Those miRNAs that are altered in plasma before the first spontaneous seizure, like miR-9a-3p, may be proposed as putative biomarkers of epileptogenesis.

Dysregulation of the (immuno)proteasome pathway: common pathologic hallmark of experimental and human focal epilepsy

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Purpose: The proteasome is a multisubunit enzyme complex involved in protein degradation, which is essential for many cellular processes. Under inflammatory conditions, the constitutive subunits are replaced by their inducible counterparts, resulting in the formation of the immunoproteasome, which has been suggested to play a role in epilepsy. In the present study we investigated the expression of constitutive and immunoproteasome subunits in the post-status epilepticus rat model and in human brain tissue. We also investigated whether (immuno)proteasome subunit expression could be modulated in human astrocyte cultures by the immunomodulators rapamycin and curcumin.

Methods: Immunohistochemistry of β1, β1i, β5 and β5i subunits was performed 1 day (acute phase, n=5), 1 week (latent phase, n=6) and 7 months after status epilepticus (chronic phase, n=13) that was induced in rats via electrical stimulation of the angular bundle. The number of immunoreactive cells in the hippocampus and their immunoreactivity were estimated using semi-quantitative analysis and the immunoreactivity score (IRS: number of cells * immunoreactivity) was calculated.

To study the situation in humans, a cohort of surgical specimens from patients with drug-resistant epilepsy was also included: hippocampal sclerosis (HS, n=20; ILAE type 1), non-HS (n=16), malformations of cortical development, including mild malformations of cortical development (mMCD, n=6), focal cortical dysplasia (FCDIIa, n=5; FCDIIb, n=6) and cortical tubers from patients with Tuberous Sclerosis Complex (TSC, n=6). To mimic the situation of an initial precipitating event we also included patients that died after

traumatic brain injury (TBI, n=15) or after status epilepticus (SE, n=6). All findings were compared to age-matched autopsy controls (n=14). Furthermore, primary human astrocyte cultures stimulated with interleukin 1β (IL-1 β) were used to study the effects of 100 nM rapamycin and 10 µM curcumin on (immuno)proteasome subunit expression.

Results: In rats, the IRS of β1i and β5 was higher in granule cells and pyramidal cells of the hippocampus during the acute phase compared to controls. At this time point, the IRS of β5 was also higher in astrocytes within the hippocampus. During the latent and chronic phases, the IRS of β1, β1i, β5 and β5i was higher in granule cells and pyramidal cells and/or astrocytes compared to controls. Interestingly, in rats which developed a progressive form of epilepsy the IRS of β1i and β5 in granule cells and β5i in astrocytes was higher than in rats with a non-progressive form.

In human temporal lobe epilepsy, the IRS of β1, β1i, β5 and β5i was higher in hippocampal neurons and astrocytes compared to controls and was most pronounced in patients with HS. Similarly, in SE, TBI and mMCD the IRS of all subunits was higher in neurons and/or astrocytes compared to controls. The most prominent changes were observed in TSC and FCD, in which the IRS of all subunits was higher compared to controls in astrocytes and dysmorphic neurons (FCD and TSC), balloon cells (FCDIIb) and giant cells (TSC), particularly in the nucleus of these cells.

In vitro studies using cultured human astrocytes showed that the expression of all subunits increased in the cytoplasm and particularly around the nucleus after an inflammatory challenge with IL-1β. Additionally, rapamycin attenuated gene expression of all subunits and curcumin specifically attenuated expression of the immunoproteasome subunits after IL-1β stimulation.

Conclusions: Our data suggest that dysregulation of the proteasome system represents a common pathologic hallmark of experimental epilepsy and human focal lesions associated with drug-resistant epilepsy. The expression of the (immuno)proteasome is linked to the severity of pathological alterations and epilepsy and can be modulated with rapamycin and curcumin. Therefore, this system may represent an interesting novel target for drug treatment in epilepsy.

Development of Radiolabeled Tracers for Imaging of Epileptogenesis

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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 and the underlying mechanisms of epileptogenesis. On the basis of discussions during previous Epitarget meetings, we focused our research on four different targets involved in neuroinflammation (Figure 1).

Structural changes at the blood-brain barrier (BBB) are believed to play a crucial role in epileptogenesis. Breakdown of the BBB results in infiltration of human serum albumin (HSA) and other proteins in the brain. In order to monitor this process, we have prepared a dually labeled HSA tracer bearing a radioisotope and a fluorescent tag.

Increased expression of vascular cell-adhesion molecule 1 (VCAM-1) at the endothelial cells of the BBB can potentially indicate functional changes of this barrier. We have previously reported a radiolabeled VCAM-1 antibody coupled to iron oxide microparticles for imaging of VCAM-1. Moving forward, a new multimodal peptidic tracer is being developed, based on a well established VCAM-1 recognition sequence and a bio-compatible backbone.

Increased expression of the metabotropic glutamate receptor 5 (mGluR5) has been observed in several neuronal diseases, including epilepsy. [18F]-FPEB is one of the most promising mGluR5 tracers; however, the use of this tracer has been limited to a few pilot studies due to the highly challenging synthesis. We have recently developed a new labeling strategy based on sulfonium salts as leaving groups that allows us to efficiently prepare [18F]-FPEB in high radiochemical yields, making this tracer available for preclinical and clinical applications.

Monoamine Oxidase-B (MAO-B) is a biomarker that reflects astroglial activation. Imaging of MAO-B has been reported using 11C-L-deprenyl; however, the practical use of this

tracer is limited by the short half-life of carbon-11 (20 min). We aim at synthesizing [18F]-fluorodeprenyl using the sulfonium salts chemistry mentioned above.

The current status of each tracer development project, as well as future directions, will be discussed.

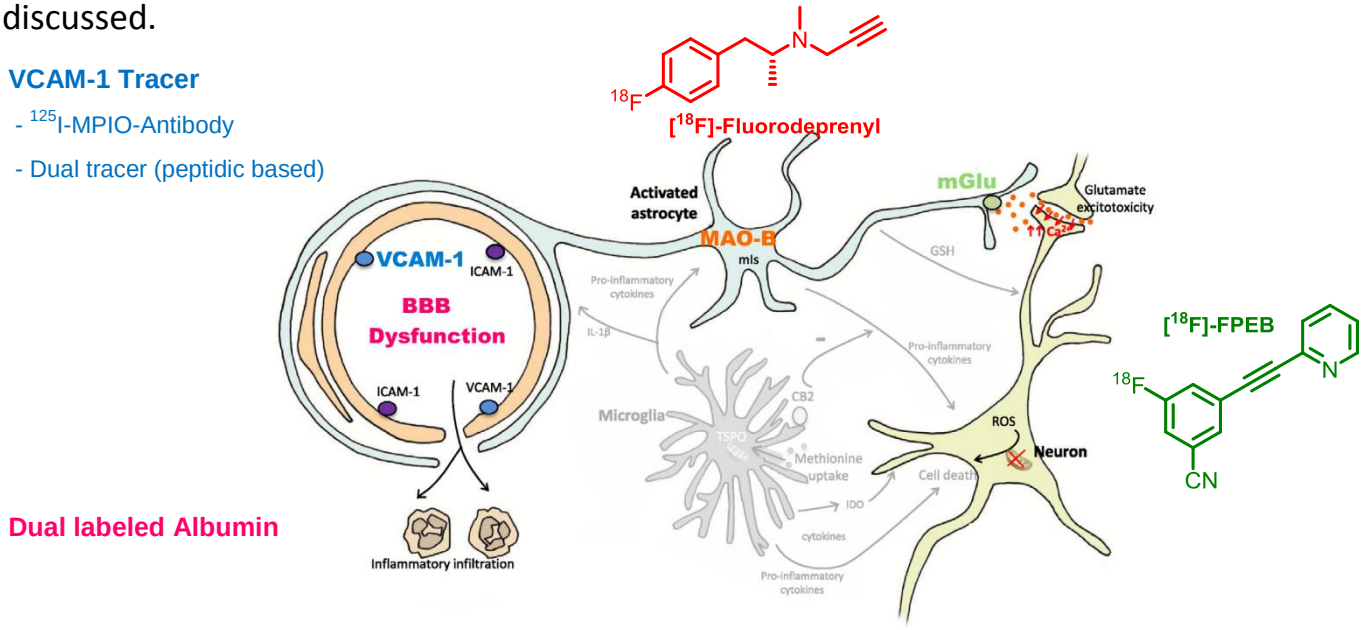

Figure 1: Radiotracers in development at University College London to image epileptogenesis. Adapted from Amhaoul et al., Neuroscience (2014)

Mechanisms of resolution of neuroinflammation in epileptogenesis

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Rationale. Neuroinflammation is a major hallmark of pharmacoresistant epilepsy with a highly suspected pathological role. It is induced by various epileptogenic injuries and sustained by seizures. Neuroinflammation contributes to acute and chronic seizures in experimental models, and possibly to epileptogenesis, likely because the diseased brain lacks efficient pro-resolving/anti-inflammatory mechanisms for limiting its extent and duration. It is, therefore, important to identify key regulators of neuroinflammation in epilepsy for promoting its fast and efficient resolution thus preventing the deleterious consequences of uncontrolled and persistent inflammation.

We present evidence on two distinct targets to boost resolution mechanisms: a) pro-resolving lipids; b) microRNA-146a that inhibits a major pro-inflammatory and ictogenic signal (IL-1R/TLR pathway).

Methods. a) Status Epilepticus (SE) was induced in adult male mice by intracerebral kainate or systemic pilocarpine. Mice were sacrified at different time points after SE to assess the levels of pro-inflammatory cytokines and key biosynthetic enzymes of pro-resolving lipids by RT-PCR. BML111, a pro-resolving lipoxin analogue, was injected intracerebroventricularly in mice after kainate-induced SE, and its effect was assessed by immunohistochemistry on pro-inflammatory cytokines expression in the hippocampus, 48 h post-SE. b) Either acute self-remitting seizures or chronic spontaneous seizures were induced by intracerebral injection of kainate in mice chronically implanted with hippocampal electrodes. Seizures were monitored and quantified by EEG analysis. A synthetic analog of miR-146a (mimic) or its respective negative control was injected intracerebroventricularly. We carried out a transcriptomic analysis in the hippocampus to determine which set of genes were affected by the mimic treatment. Western blot was used to measure protein levels.

Results. a) The expression of specific pro-resolving molecules was limited to pyramidal neurons and hilar interneurons in sham mice while it was induced in activated astrocytes 72 h after SE. Pro-resolving molecules were induced with a delayed time-course as compared to pro-inflammatory mediators. Treatment with BML111 abolished the SE-induced increase in pro-inflammatory cytokines expression measured during epileptogenesis. b) The miR-146a mimic injection induced a transient 4-fold increase in miR-146a forebrain levels and a concomitant reduction in IL-1R/TLR signalling proteins. The mimic significantly reduced neuronal excitability and acute seizures. Moreover, the mimic blocked disease progression when injected in mice after epilepsy onset, and reduced cell loss and improved memory deficit when given shortly after SE. These therapeutic effects were associated with broad modifications in immune/inflammatory pathways in the brain.

Conclusions. This evidence reveals that boosting pro-resolving/anti-inflammatory mechanisms during epileptogenesis or at the initial phases of the disease may represent an effective strategy to prevent the deleterious consequences of neuroinflammation, therefore, may offer a novel therapeutic option for neuroprotection and disease-modifications in epilepsy.

Memory performance in TLE: Future prognostic biomarkers

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Temporal lobe epilepsy (TLE) is a severe brain disorder affecting particularly young adults. Often, TLE is associated with memory deterioration and hippocampal selective neuronal impairment. It thereby shows striking parallels to neurodegenerative disorders including Alzheimer's disease (AD). Using human hippocampal tissue derived from epilepsy surgery (n=79) we established a genome wide expression array analysis, which provides differential hippocampal gene expression patterns in patients with very severely versus average memory performance. We found neuronal molecules to be abundantly expressed in patients with epilepsy and major impairments in memory performance. Subsequent promoter analysis revealed the single nucleotide polymorphism rs744373 C-allele to be associated to high mRNA levels of bridging integrator 1 (BIN1), a crucial genetic AD risk locus that plays an important role in transient calcium potentials, apoptosis, trafficking and clathrin-mediated endocytosis. Using in vitro luciferase transfection assays, we found that BIN1 promoter activation is genotype dependent and strongly reduced by binding of the transcription factor TGIF. Our results give new insights on functional genetic influences on memory performance in TLE patients, which may characterize new biomarkers for future drug screening and be extrapolated to other neurodegenerative disorders.

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Development of an in vitro brain autoradiography platform

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In vitro autoradiography is a powerful technique for quantitative assessment of receptor binding. In addition to in vivo molecular imaging, it is a valuable complementary approach due to its higher spatial resolution and the opportunity of parallel evaluation of different targets. Here, we describe a standardized autoradiography platform to study microglia activation in brain slices during epileptogenesis.

Native brain tissue slices of 14 µm are cut on a cryostat and mounted on permanently positively charged standard microscope slides. Slides are incubated in a solution containing 20 kBq/ml of a translocator protein (TSPO) tracer. After drying, slides are exposed to a phosphor-imaging film for 15 min. Once the exposition is finished, the film is scanned and digitalized (Cyclone Plus, Perkin Elmer). Analysis is performed in Pmod software, overlaying a predefined ROI atlas to each slice. To normalize autoradiographies, cerebellar slices from the same naïve animal are co-incubated and analyzed within every experiment. Furthermore, an individual activity calibration curve is set up for each experiment and used for absolute quantification.

In vivo TSPO-PET signals before and 5 days after status epilepticus correlated well with in vitro autoradiography (R^2 = 0.99317) in epileptogenesis-associated brain regions (amygdala, hippocampus, thalamus). In addition, both normalization approaches showed consistency in experiments with different times of exposition or incubation. Calibration curves correlated well with signal density obtained after exposing slices to the film, resulting in R^2 values of 0.96 to 0.99.

With this platform, we can perform reliable quantitative in vitro autoradiography with different radiotracers, for obtaining information about different receptors and their possible correlation in epileptogenesis. In addition, this platform will allow us to study different models of epileptogenesis by performing autoradiography with slices provided by collaborating groups, enabling a direct comparison between different epileptogenesis

models. Furthermore, the platform can be used for evaluation of surgically removed or post mortem patient tissue.

Behavioral characteristics of the rat amygdala stimulation model of temporal lobe epilepsy

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Epilepsy is one of the most common neurological diseases, affecting around 1% of the World's population. It causes high financial burden for medical care systems and sociological issues for patients. In some patients epilepsy can be linked to brain injury, stroke or other insult to the brain. I such cases epilepsy frequently develops after long latency period. Currently, prediction of which patient eventually develops epilepsy is not possible as there are no biomarkers available. In this work we performed in-depth behavioral characteristic of amygdale stimulation model of epilepsy to find early signs of epileptogenesis/epilepsy that could be used as behavioral biomarkers in preclinical studies. We performed the battery of behavioral tests, including: Open Field, New Object Recognition, Elevated Plus Maze and the Morris Water Maze, at the early (1 month after stimulation) and late time points (6 months after stimulation). We identified changes in performance of amygdala stimulated rats, at both time points studied. Correlation of behavioral indices with EEG and molecular findings is ongoing.

Assessment of novel combinations of phenotypic biomarkers to predict development of epilepsy in the lithium-pilocarpine model of temporal lobe epilepsy in rats

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The goal to develop antiepileptogenic interventions would be greatly facilitated by the identification of reliable biomarkers of epileptogenesis. Given the complexity of epilepsy, it is unlikely that a single biomarker is sufficient for predicting epileptogenesis, but a

combinatorial approach may be necessary to identify appropriate biomarkers at different stages of the evolution of the disease. Such combinatorial biomarker approaches are currently being explored in Alzheimer's disease, traumatic brain injury, and brain cancer, and may overcome the challenge of individual variability and disease heterogeneity, but as yet little is known about the utility of biomarker combinations to accurately predict epilepsy after brain insults. We recently started to investigate phenotypic biomarkers of epileptogenesis in different post-status epilepticus (post-SE) mouse and rats models of temporal lobe epilepsy (TLE). One of these phenotypic biomarkers is seizure threshold, which is thought to determine the propensity or likelihood for a seizure to occur and is decreased after different types of epileptogenic brain insults, although not all animals with decreased seizure threshold develop epilepsy. Other potential phenotypic biomarkers are cognitive and behavioral alterations that occur early after an epileptogenic brain insult and can be assessed by a battery of behavioral tests. The goal of the present prospective study in the lithium-pilocarpine model of TLE in rats was to determine the discriminative utility of combinations of such phenotypic biomarkers by examining their ability to predict epilepsy. For this purpose, we used a recent model refinement that allows comparing rats that will or will not develop spontaneous recurrent seizures (SRS) after SE (Brandt et al., Neurobiol. Dis. 2015). Seizure threshold was determined by timed i.v. infusion of pentylenetetrazole (PTZ) before and at 1, 2 and 3 weeks after SE. Behavioral alterations were assessed by a test battery one day before each PTZ infusion. Three and 6 months after SE, continuous (24/7) video-EEG monitoring was used to determine which rats had developed SRS. After all experiments were completed, three groups of rats were compared: (1) sham controls; (2) post-SE rats without SRS; and (3) post-SE rats with SRS. All data were both assessed alone and in combination, and operating characteristic (ROC) curve analyses were performed to determine whether a biomarker or combination of biomarkers performed better than chance at predicting epilepsy after SE. When the area under curve (AUC) of ROC curve analyses was compared with chance (AUC = 0.5), AUC from combined data was 0.9592, indicating an almost perfect discrimination or accuracy to predict development of SRS. Such high AUC was not reached with any of the potential biomarkers alone. To our knowledge, this is the first study that indicates that combinatorial biomarker approaches may overcome the challenge of individual variability in the prediction of epilepsy.

Epileptic Seizures Induce an Immune Response in the Rat Eye

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Epileptic seizures are characterized by a number of pathological hallmarks, such as an imbalance in synaptic transmission and immune response. However, it is currently not know whether these seizure-induced alterations can propagate to areas outside cortical/subcortical structures. We therefore investigated whether status epilepticus induces an immune response in the retina, a remote extension of the brain. Adult rats underwent electrically-induced temporal status epilepticus and eyes were studied acute (6hrs), sub-acute (1w) and late (7ws) after seizures. Biochemical analyses revealed unaltered expression of cytokines and chemokines at 6hrs compared to non-stimulated controls. At 1 and 7ws, retinal cytoarchitecture appeared normal and the number and morphology of microglial cells was unaltered at 1w. However, at 7ws, numbers of microglia were increased in the retina, both ipsi- and contralateral to the epileptic focus. They remained located within plexiform layers, but often in clusters and with more processes in the outer nuclear layer. The percentage of ramified microglia was decreased, while amoeboid morphology was increased. No alterations were observed in numbers of phagocytic cells, infiltrating macrophages, or vascular pericytes. Instead, astrocytes and Müller (or macroglial) cells, exhibited more and longer processes. Post-synaptic density-95 cluster intensity was reduced in the outer nuclear layer, which reflects seizure-induced synaptic changes in the area with increased microglial activation. Interestingly, intracerebroventricular infusion of CX3CR1 antibody, known to reduce microglial activation in the epileptic focus, reduced micro-and macroglial activation in the retina.

The present results are the first evidence that seizures induce an immune response in the eyes. As it is a structure more easily accessible for non-invasive evaluations, it may become an attractive biomarker and diagnostic tool to study brain inflammation.

Collagen VI Modulates Synaptic Transmission in the Hippocampus

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Collagen VI (CVI) is a member of the collagen family proteins primarily known as components of the extracellular matrix with a structural and functional role in connective tissues. It has been recently reported that CVI mRNA and protein levels are increased in the hippocampus of an animal model for Alzheimer's Disease (AD), protecting neurons against Aβ toxicity (1). Since AD is concomitant to epilepsy (2), we wondered whether CVI is involved in epileptogenesis and/or have a functional effect in synaptic transmission.

References: (1) Cheng et al., Nat Neurosci 2009; 12(2):119-21. (2) Palop and Mucke. Arch Neurol 2009; 66(4):435

Characterisation and tuning of the intrahippocampal kainate model of temporal lobe epilepsy.

Esbjörn Melin

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In order to successfully develop new treatments for temporal lobe epilepsy (TLE), we need models that better mimic the disease, but also represent those individuals that don't develop spontaneous seizures after insults, for comparison of biomarkers. The intrahippocampal kainic acid (KA) model shares many features of the human chronic epileptic condition, but further characterization of the model is needed. Here we used an i.h. bolus dose of KA followed 24/7 video-EEG monitoring during epileptogenesis and the early chronic state. The first electrographical seizure was detected on average 12.3 days post KA injection and there was a big variation in the spontaneous seizure frequency (8.91± 2.21 seizures per week). Also, only 38 % of the animals developed seizures. In another batch of animals we changed the preparation of kainic acid and shortened the surgery time achieving chronic seizures in over 80% of the animals. This suggests that the rate of animals developing seizures can be tuned to benefit the experiments.

Delayed activation of pro-resolving mechanisms during epileptogenesis in mice

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Rationale. Neuroinflammation is a major pathological factor in epilepsy. It is induced by experimental epileptogenic injuries and seizures as well as in human pharmacoresistant epilepsy. Neuroinflammation contributes to acute and chronic seizures, and possibly to epileptogenesis, likely because the diseased brain lacks efficient anti-inflammatory mechanisms for limiting its extent and duration. Resolution of inflammation is a highly coordinated process chiefly controlled by endogenous pro-resolving lipids and peptides. Our hypothesis is that neuroinflammation is inefficiently controlled by pro-resolving mediators, such as ResolvinD1 (RvD1), LipoxinA4 (LXA4), AnnexinA1 (AnxA1) and their receptors ChemR23 and LXA4 Receptor (ALXR). We studied, therefore, the expression of pro-resolving molecules during epileptogenesis induced by status epilepticus (SE) in mice, and validated the presence of some molecules in hippocampi from patients with mesial temporal lobe epilepsy (TLE). The final goal is to limit the pathologic consequences of the inflammatory response by boosting its resolution with pharmacological interventions.

Methods. SE was induced in adult male mice by intra-amygdala kainate or systemic pilocarpine. Mice were sacrified 2 h, 24 h, 72 h and 1 week after SE. The pro-inflammatory cytokines IL-1β and TNF-α, ChemR23 and ALXR, and key biosynthetic enzymes of pro-resolving lipids LOX-5 and LOX-15 were analyzed by RT-PCR and immunohistochemistry in the hippocampus of SE and sham mice. Some molecules were analyzed also in the hippocampus of TLE patients and autopsy controls. BML111, a LXA4 stable analogue, was injected intracerebroventricularly in mice after kainate-induced SE, and its effect was assessed by immunohistochemistry on IL-1β and TNF-α expression in the hippocampus, 48 h post-SE.

Results. ChemR23, ALXR and AnxA1 protein expression was limited to pyramidal neurons and hilar interneurons in sham mice. 72 h after SE, they were induced in activated

astrocytes. LOX-5 and LOX-15 mRNA levels were also up-regulated 72 h after SE in the hippocampus of mice and of TLE patients. These protein alterations after SE were similar both in kainate and pilocarpine mice, thus excluding model-specific effects. Pro-resolving molecules were induced with a delayed time-course compared to inflammatory cytokines. Treatment with BML111 virtually abolished the SE-induced increase in IL-1β and TNF-α expression.

Conclusions. Pro-resolving mediators are induced after SE likely reflecting seizure-mediated up-regulation. These molecules are also induced in TLE patients suggesting that pro-resolving signaling activation occurs in pharmacoresistant epilepsy. Neuroinflammation following SE precedes the up-regulation of pro-resolving mechanisms denoting a delayed activation of resolution mechanisms during epileptogenesis. Treatment with a drug mimicking LXA4 strongly reduces neuroinflammation. This evidence reveals that pharmacological intervention with pro-resolving drugs reduces neuroinflammation, thus supporting further studies on their potential anti-epileptogenic actions.

miR-146a-based therapy against neuroinflammation has anti-ictogenic and disease-modifying effects in murine models of seizures and epilepsy

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Aim: Our goal was to study the anti-ictogenic and anti-epileptogenic properties of miR146a that modulates the activation of the IL-1R/Toll-like receptor signaling, a major neuroinflammatory pathway activated both in experimental and human epilepsy.

Methods: Either acute self-remitting seizures or chronic spontaneous seizures were induced by intracerebral injection of low or high doses of kainate in mice chronically implanted with hippocampal electrodes. Seizures were monitored and quantified by EEG analysis. A synthetic analog of miR146a (mimic) or its respective negative control was injected intracerebroventricularly using different protocols depending on the model. We carried out a transcriptomic analysis in the hippocampus to determine which set of genes were affected by the mimic treatment. Western blot was used to measure protein levels.

Results: The miR146a mimic injection induced a transient 4-fold increase in miR146a forebrain levels and a concomitant reduction in IL-1 receptor/Toll-like receptor signalling proteins. The mimic significantly reduced neuronal excitability and acute seizure susceptibility. Moreover, the mimic blocked epilepsy progression when injected in mice after the disease onset, and reduced cell loss and improved memory deficit when given shortly after status epilepticus. These therapeutic effects were associated with broad modifications in immune/inflammatory pathways in the brain.

Conclusions: We conclude that miR-146a is a putative therapeutic agent for neuroprotection and disease-modifications in epilepsy, and possibly in neurological diseases with a pathologic neuroinflammatory component.

Characterization of the pilocarpine model of epileptogenesis by serial molecular in vivo imaging

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Rationale: The pilocarpine post status epilepticus (SE) model is a widely used rat model of epileptogenesis that reflects brain pathology of insult-induced epilepsies in many aspects. Particularly, it reflects brain inflammation and blood-brain barrier alterations, which are

suggested to be key processes mediating insult-induced epileptogenesis. Non-invasive molecular imaging may (i) identify these processes as epileptogenesis biomarkers that hold potential for translation to the clinic, (ii) help to define appropriate time windows for anti-inflammatory and BBB-protective, epilepsy preventing pharmacotherapy, and (iii) function as a tool to survey treatment effects.

Methods: To evaluate the time course of cerebral inflammation and BBB leakage during epileptogenesis, serial positron emission tomography (PET)/CT was performed in the rat lithium-pilocarpine post-status epilepticus (SE) model using (i) F-18-FDG to investigate glucose metabolism, (ii) translocator protein (TSPO) ligand C-11-PK11195 to detect microglial activation, (iii) Ga-68-DTPA to investigate BBB integrity, (iv) F-18-FET for protein metabolism, and (v) C-11-verapamil for activity of the multidrug transporter P-glycoprotein. Furthermore, T2 and gadolinium-DTPA-enhanced T1 MRI was performed to evaluate structural brain changes like cell edema and leakage of the BBB. Brains were subsequently processed for complementary autoradiography and histological analyses.

Results: F-18-FDG PET revealed regional hyper-glucometabolic changes only directly associated with the epileptogenic insult (first 24 h) indicating increased neuronal and astroglial activity, whereas hypometabolism was present for both glucose and proteins during chronic epilepsy (10 w post SE), a phase which is characterized by decreased hippocampal volume and enlarged ventricles detected by MRI. BBB leakage was observed as early as 5 h post SE (FITC-albumin extravasation) in typically SE-affected brain regions, remained present for at least 48 h post SE (FITC-albumin extravasation, increased T1-MRI signal), but was not detected any more at 10 d post SE. In addition, an increase in P-glycoprotein transport activity was present at 48 h. In parallel, cerebral edema reflected by an increase in T2-MRI signal was observed 24 and 48 h post SE. Microglial activation first appeared 48 h, peaked about 7 d post SE, and was subsequently decreasing over the following weeks. The time profile of microglial and astroglial activation as analyzed by immunohistochemistry corresponded to that demonstrated by C-11-PK11195-PET.

Conclusions: Brain regions commonly associated with seizure generation display distinct time profiles of epileptogenesis-associated patho-mechanisms. Based on these profiles, first anti-inflammatory and BBB-stabilizing treatment approaches are currently evaluated.

Refinement of the intrahippocampal kainate model of epilepsy in rats

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A large variety of brain insults, including a status epilepticus (SE), can induce the development of symptomatic epilepsies, particularly temporal lobe epilepsy (TLE). In the latent period after the initial insult multiple molecular, structural, and functional changes, called epileptogenesis, proceed in the brain. All these mechanisms can lead to spontaneous recurrent seizures (SRS). An urgent medical need is to develop antiepileptogenic strategies that modify or even prevent the development of SRS.

In rodent post-SE models, kainate, a neurotoxic glutamate analogue, is widely used to induce SE for the investigation of epileptogenesis and TLE. Focal injection of kainate in the hippocampus induces an epileptic focus and alterations in the brain that are similar to human TLE.

In mice, the chronic epileptic state in the focal kainate model is characterized by a high frequency of subclinical seizures in EEG of the ipsilateral hippocampus and infrequent secondarily generalized convulsive seizures. In rats, only infrequent focal and secondarily generalized convulsive seizures are observed. The rat model is therefore much more time-consuming and less suitable for investigating the antiepileptogenic potential of different drugs and their combinations. The reason for this species difference is not known, but the dose of kainate and the localization of kainate injection in the hippocampus may be involved. For this reason we modified the focal kainate model in rats and adapted it accordingly to the focal kainate model in mice. We injected kainate with or without anaesthesia, implanted an electrode in the hippocampal focus and changed step by step the injection site, the dose, and the volume of the kainate injection.

Results indicate that neither the change of injection site in the hippocampus nor the increase of the kainate dose or volume lead to similar electrographic seizure frequencies as seen in mice. A further species difference was that intrahippocampal kainate induced SRS in anesthetized mice, whereas aesthesia suppressed development of SRS in rats, so that kainate had to be injected in conscious rats. These findings indicate that it is not possible to mimic the advantages of the mouse model in rats, and therefore it remains

more time-consuming to study antiepileptogenic effects in the intrahippocampal rat model. We will therefore use this model only as second stage of our 2-stage approach of evaluating novel combinations of clinically used drugs for antiepileptogenic efficacy.

Analysis of *omics data in TLE brain tissue

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As part of the EPITARGET Project the UKMB comprises one of the world's largest fresh frozen biobanks of human bioptic hippocampus specimens from epilepsy surgery of pharmacoresistant patients. The UKMB has a transgenic platform for the generation of 'viral transgenic' mice and in addition a neuropathology platform with long standing expertise in epilepsy associated lesions in humans and experimental animals. The project will be complemented by the central "*omics platform" of L&B. Here, exome sequencing, RNA-sequencing, microRNA- and promoter methylation analyses are currently launched of 55 fresh-frozen hippocampi sections and corresponding blood from pharmacoresistant TLE patients that underwent epilepsy surgery for seizure control. DNA, RNA and microRNA (miRNA) is isolated from hippocampi tissue, as well as patient corresponding DNA from EDTA blood. If available a blood sample from the day of surgery (DOS) is taken for DNA isolation, otherwise blood from another time point is taken. DNA from EDTA blood is used for exome sequencing, a next generation sequencing (NGS) technique that will sequence all protein coding genes in the genome. The goal is the identification of genetic variation in epileptic patients compared to a reference genome. DNA isolation from fresh-frozen hippocampus tissue sections will be used for methylation analysis, to potentially elucidate epigenetic alterations in gene expression. Total RNA isolated from the same hippocampi sections will be used for RNA-sequencing (RNA-seq) and miRNA analyses. RNA-seq is a powerful NGS method to get an insight on alternative spliced gene transcripts, possible gene fusions, post-transcriptional modifications, single nucleotide polymorphisms (SNPs)/ mutations and general changes in gene expression. Finally, miRNA-analyses is performed

to characterize the expressed miRNAs in hippocampi of different cohorts of pharmacoresistant epilepsy patients.

After generating these datasets emphasis will be given on system biology approaches to discover genetic networks that correlate with chronic epilepsy outcomes. Altogether, this will help to identify and characterize particular long-term, stable biomarkers in human chronic epilepsy.

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Controlling Epileptiform Activity with 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 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.

Sestrin 3 as a regulator of a proconvulsant gene network

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Epilepsy is a serious neurological disorder that affects 1% of the world's population. Several recent studies have implicated Toll-like receptor (TLR) signaling and release of proconvulsant inflammatory molecules (i.e. interleukine 1β) in both seizures generation and epileptogenesis (Vezzani et al., 2000; Maroso et al., 2010; 2011). Here we employed systems genetic approaches to characterize the genetic regulation of pathophysiological pathways in TLE. We identified a gene-regulatory network genetically associated with epilepsy that contain a specialized, highly expressed transcriptional module encoding proconvulsive cytokines and TLR signaling genes. We uncovered pathways and transcriptional programs associated with epilepsy that are conserved in the mouse epileptic hippocampus. Using genome-wide Bayesian expression QTL mapping, we probed the genome for key genetic regulators of the network in the human brain. We pinpointed the unexpected gene, Sestrin 3 (SESN3) whose protein product controls the intracellular response to reactive oxygen species (Budanov et al., 2004; Nogueira et al., 2008; Hagenbhchner et al., 2012; Zamkova et al, 2013), as a trans-acting genetic regulator of the proconvulsant gene network in the human epileptic hippocampus. We carried out validation experiments in independent in vitro and in vivo systems, demonstrating that SESN3 positively regulates the module in macrophages, microglia and neurons; moreover, morpholino-mediated SESN3 knockdown in zebrafish confirmed the regulation of the transcriptional module, and attenuated chemically induced behavioral seizures. SESN3 knock out rats have been created from the Sprague-Dawley strain and their epileptic phenotype has been investigated using the pilocarpine model. Our results shown that SESN3 silencing increases the time needed to enter status epilepticus after pilocarpine injection, and that an higher dose of the convulsant is necessary to elicit status epilepticus. We also studied the phenotype of this new strain of rats using different behavioral tests, including elevated plus maze, novel object recognition, open field and forced swimming. This behavioral analysis revealed that SESN3 KO rats are less anxious and less prone to develop depression compared to control Sprague Dawley rats. Taken together, these data provide the first evidence of a function for SESN3 in regulating endogenous proconvulsant factors (for example, TNF-a, IL-1 and TLR-signaling genes) in the human epileptic hippocampus, and suggest SESN3 as a new potential target for modulating brain inflammation and excitability. Our systems genetics approach builds on

and extends previous methods correlating individual genetic variations with disease susceptibility by identifying disease-associated gene networks, pathophysiological pathways and their upstream genetic regulators. More in general, this systems genetics framework can be employed to identify genes and regulatory networks across diverse neuropsychiatric disorders where genetic factors can perturb underlying molecular pathways in the brain.

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