

Projekte:

Teilprojekt D6 im Transregio Sonderforschungsbereich TR3 "Mesiale Temporallappen-Epilepsien"

"Role of epileptic activity and Reelin in neurogenesis in the dentate gyrus"

Previous studies have provided evidence for a stimulatory role of epileptic activity in adult neurogenesis. Thus, intraperitoneal (i. p.) injection of kainate or pilocarpine resulted in increased neurogenesis of dentate granule cells (Hüttmann et al., 2003; Gong et al., 2007). In contrast, intrahippocampal kainate injection, which like i. p. injection of kainate or pilocarpine induces epileptic activity, resulted in the cessation of neurogenesis on the injected side but not on the contralateral side (Heinrich et al., 2006). Cessation of neurogenesis was accompanied by glia hypertrophy and a decreased expression of the extracellular matrix molecule Reelin. In reeler mutants lacking Reelin, neurogenesis was similarly found decreased, whereas gliogenesis was increased, likely due to a precocious differentiation of radial glial cells to astrocytes (Zhao et al., 2007). Together, these findings indicate that epileptic activity alters Reelin expression which in turn may affect the balance between neurogenesis and gliogenesis in the dentate gyrus. The present project aims to elucidate the roles of epileptic activity and Reelin in neurogenesis in the dentate gyrus. We will ask: Under which conditions does epileptic activity stimulate or decrease neurogenesis? Is decreased neurogenesis after hippocampal kainate injection caused by decreased Reelin expression? Does decreased Reelin expression in epilepsy cause an increased gliogenesis, similar to the increased gliogenesis in reeler mutants? Is Reelin important for cell fate decisions of newly generated cells in the dentate gyrus? Can neurogenesis be stimulated by Reelin? To approach these questions, we will use the model of intrahippocampal kainate injection and slice cultures of hippocampus. We will study the effects of kainate application on Reelin expression and neurogenesis/gliogenesis and mimic decreased Reelin expression by using function blocking antibodies. We will study Reelin effects on the signaling of Notch1, known to be important for the maintenance of undifferentiated progenitor cells. We expect these studies to contribute to our understanding of the molecular changes associated with epileptic activity and their consequences for adult neurogenesis/gliogenesis in the hippocampus.

Zeitraum: 7/2008 - 6/2012 Antragsteller: Haas, Frotscher

Teilprojekt D7 im Transregio Sonderforschungsbereich TR3 "Mesiale Temporallappen-Epilepsien"

"Experimentally induced granule cell dispersion as a model to study the role of migration defects in epilepsy"

Cortical migration defects are often associated with the occurrence of epilepsy. Also in temporal lobe epilepsy (TLE), a migration defect of dentate granule cells, termed granule cell dispersion (GCD), is frequently observed. Little is known how GCD develops and to which extent it contributes to the development of seizure activity. We have previously shown in TLE patients and in a mouse TLE model that the development of GCD correlates with a loss of the extracellular matrix protein reelin (Haas et al., 2002; Heinrich et al., 2006). In addition, we showed that GCD occurs in the absence of neurogenesis, thus representing a displacement of mature neurons due to a reelin deficiency (Heinrich et al., 2006; Fahrner et al., 2007). Accordingly, antibody blockade of reelin function in naïve, adult mice induced GCD (Heinrich et al., 2006). In this grant proposal we want to study whether (1) pathological processing of reelin contributes to GCD formation and if so, can we reverse it; (2) whether we can rescue GCD by in vivo infusion of reelin and thereby reduce seizure activity and (3) whether GCD causes disturbances of input connections and thus contributes to the formation of an imbalanced network. In addition, we will induce GCD by neutralization of reelin in the adult, healthy mouse hippocampus and analyze whether this disturbed lamination results in seizure activity.

Zeitraum: 7/2008 - 6/2012 Antragsteller: Haas, Frotscher Projektleiterin: C. Haas

Teilprojekt C2 im Sonderforschungsbereich 780 "Synaptic Mechanisms of Neuronal Network Function"

"Signal integration in the dentate gyrus under physiological and pathophysiological conditions"

The strict laminar organization of the hippocampus is a striking feature, but its functional relevance is not clear. Unilateral injection of kainate (KA) into the hippocampus of mice produces Ammon's horn sclerosis (AHS), which is accompanied by extensive re-organization of the dentate gyrus network: the lamination of granule cells (GCs) is lost, specific interneurons die and GC axons sprout back into the GC input region. By comparing this pathological network with the physiological situation we will study the relevance of GC morphology and input organization for signal integration in GCs. Since GCs have been suggested to play a role in processing epileptic seizure signals and since AHS is a major symptom of temporal lobe epilepsy, this project is also suited to reveal the role of GCs in seizure generation. Initially, we will investigate whether the intrinsic properties of GCs are changed in KA-injected mice and what the consequences for input-output integration are. Recorded neurons will be filled with biocytin and 3-D reconstructed for quantitative morphological analysis using ApoTome technique and Imaris software. To evaluate the relationship between morphological and electrophysiological parameters, we will assess the spatiotemporal profile of morphological and functional changes at different time points after KA injection. Ionic mechanisms responsible for altered electrophysiological properties of GCs will be pharmacologically characterized. We will use laser microdissection combined with real time reverse transcription polymerase chain reaction (RT-PCR) to analyze expression of ion channel candidates. To test whether the impact of the segregated input is altered in AHS, we will stimulate in the different sub-regions of the dentate molecular layer and assess the input-specific synaptic plasticity. Paired recordings of GCs and basket cells will be performed to establish whether proximal inhibition of GCs is changed in AHS and how this affects integration of distal inputs. To analyze the synaptic mechanisms in more detail, we will use infrared-guided transmitter uncaging to differentially activate sub-groups of postsynaptic receptors. The results of these experiments will be correlated at the single-cell level with the distribution of postsynaptic receptor subtypes or ion channels determined by immunocytochemistry. To elucidate whether pathological spine formation is associated with new synapses, we will analyze biocytin-labeled GCs by electron microscopy. Possible changes in afferent connectivity will be characterized by retrograde and anterograde tracing techniques. We believe that this interdisciplinary approach will help to understand signal integration in the dentate gyrus, in particular with respect to the integration of seizure activity. A notable advantage of this project is that we can study the relevance of potential mechanisms to humans, because we have access to human hippocampi resected during epilepsy surgery.

Zeitraum: 1/2008 - 12/2011 Antragsteller: Wolfart, Haas

Teilprojekt C1 im Bernstein Center for Computational Neuroscience/Center for neural dynamics Freiburg (BCCN) "Applications to biomedicine and new technologies"

Human temporal lobe epilepsy (TLE) is often accompanied by sclerosis in the hippocampus (HC) with neuronal degeneration and dentate gyrus granule cell (GC) dispersion. Reduced expression of reelin (a glycoprotein essential for layer formation in the brain) in TLE patients indicates its involvement in GC migration defects. How changes of the dentate gyrus microcircuitry and of the connected tissues contribute to epileptic activity is yet unclear. A new mouse model of HC sclerosis with GC dispersion enables investigations connecting structural changes and pathophysiological activity dynamics. GC dispersion in TLE is accompanied by reelin deficiency. Migration defects of GCs in reeler mice and mice without reelin receptors are associated with changes of the radial glial scaffold, the site of reelin signalling. Field potentials dynamics evoked by electrical stimulation in HC slices of epileptic mice is abnormal. Mossy fiber sprouting and recurrent innervation of GCs suggest a feedback loop promoting synchronization of spike activity, possibly associated with a deteriorating inhibitory network. We analyzed epileptiform activity in cultured hippocampal slices with substrate integrated microelectrode arrays (MEAs) and simulated HC-like neuronal networks. While previous reports focused solely on the propagation of epileptic spikes, we identified network properties important for the dynamics in the initiation phase of such spikes.

We aim to understand the mechanisms leading to TLE and Ammon's horn sclerosis by investigating the consequences of GC dispersion on network activity and to understand the relation of structural changes and pathophysiological dynamics in TLE. We will study the mechanisms leading to a decreased reelin expression associated with GC dispersion and to potential abnormalities of the radial glial scaffold in these animals.

Zeitraum: 1/2005 - 12/2009 Antragsteller: Haas, Egert, Frotscher Projektleiterin: C. Haas