distribution of synaptic depression and recovery. Those differences were relatively small and it was surprisingly difficult to correlate them with obvious changes in network activity.
Conclusions: Our results suggest that in order to understand ictogenesis we need to develop methods to accurately estimate the degree of recurrent connectivity in the network but also to measure subtle, activity-dependent inhomogeneities permissive for seizure onset. This will require detailed knowledge of both the anatomy and functional states of recurrent synaptic connectivity in epileptic networks. In theory this information would be available from activity-dependent imaging at sufficiently high temporal and spatial resolution.

1.005
A NOVEL T-TYPE CALCIUM CHANNEL ANTAGONIST DELAYS THE PROGRESSION OF EPILEPTOGENESIS IN THE AMYGDALA KINDLING MODEL OF TEMPORAL LOBE EPILEPSY
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Rationale: Temporal lobe epilepsy (TLE) is the most common form of epilepsy in adults that is refractory to medical treatment. Current therapeutic treatment is symptomatic, suppressing seizures, but has no disease modifying effect on epileptogenesis. T-type Ca2+ channels have been implicated in pathogenesis of limbic epilepsy, therefore the current study set out to investigate the effects of a novel T-type Ca2+ channel antagonist (Z944) on the progression of epileptogenesis and the treatment of seizures in the amygdala kindling model of TLE.
Methods: All female Wistar rats underwent surgery to implant a bipolar electrode into the left amygdala for electrical kindling stimulation as well as subdural electroencephalogram (EEG) recording electrodes. The anti-epileptic efficacy of Z944 was determined in fully kindled rats (five class V seizures, n=7). Z944 (5mg/kg, 10mg/kg, 30mg/kg and 100mg/kg), vehicle (0.5% Na-CMC in DMSO), ethosuximide (ETX, 100mg/kg) and carbamazepine (30mg/kg) were administered ip and 6 post-drug stimulations were given starting 15 min after drug administration. Each animal received each of the 7 treatments in a randomised manner. For the anti-epileptogenesis study, animals received Z944 (30mg/kg) (n=7), ETX (100mg/kg) (n=6) or vehicle (0.5% Na-CMC in DMSO, n=6) 30 minutes prior to each kindling stimulation up to a maximum of 30 stimulations. Animals were monitored for neurotoxicity and sedation for the entire treatment period. EEGs were analysed in a blinded manner and the kindled seizure class, primary and total seizure duration were determined. For molecular analysis, mRNA expression levels were assessed in the hippocampus and amygdala using qPCR for Cav3.1, total Cav3.2, Cav3.2 +/- exon 25 splice variants, and Cav3.3.
Results: Z944 was not effective at suppressing seizures in fully kindled rats. Z944 significantly reduced seizure class at 100mg/kg ($p<0.05$) when compared to vehicle, but this was probably due to the neurotoxicity rather than a true anti-seizure effect. For the anti-epileptogenesis study, animals receiving Z944 required significantly more stimulations to evoke a class III ($p<0.05$), IV ($p<0.01$) or V ($p<0.0001$) seizure and to reach a fully kindled state ($p<0.01$) than animals receiving vehicle. Interestingly, only one Z944 treated animal reached the fully kindled state. There was no significant difference in Cav3.1, total Cav3.2, Cav3.2 +/- exon 25 splice variants, and Cav3.3 mRNA expression in the hippocampus and amygdala between the three treatment groups.
Conclusions: These results provide evidence that T-type Ca2+ channels are important in development of limbic epileptogenesis and that drugs that target these channels may represent a new therapeutic intervention to prevent the progression of limbic epilepsy.

1.006
STRUCTURAL DIFFERENCES BETWEEN GRANULE CELLS AND SEMILUNAR GRANULE CELLS: ROLE IN DIFFERENTIAL POST-TRAUMATIC PLASTICITY OF SYNAPTIC INPUTS
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Rationale: Concussive brain trauma increases the risk for acquired epilepsy and memory dysfunction. In earlier studies, we identified that semilunar granule cells (SGCs), glutamatergic neurons in the inner molecular layer with axonal projections to CA3 (Williams et al., 2007), show enhanced excitability after brain injury. SGCs receive significantly greater inhibitory inputs than granule cells and demonstrate a post-traumatic decrease in the frequency of inhibitory synaptic inputs rather than an increase observed in granule cells (Gupta et al., 2012). Here, we examined whether differences in dendritic morphology contribute to the divergent intrinsic pattern and post-traumatic plasticity of synaptic inputs between the two cell types.
Methods: Young adult male rats were used to model brain injury. Whole-cell recordings from dentate neurons were obtained from acute hippocampal slices prepared 1 week after lateral fluid percussion injury (FPI) or sham-injury. Recorded neurons were filled with biocytin and processed for post-hoc cell identification. Neuronal reconstructions and morphometric analysis were performed on Neurolucida. Simulations done with NEURON.
Results: In contrast to the differential post-injury changes in synaptic inhibition, both granule cells and SGCs showed an increase in the frequency spontaneous EPSCs one week after FPI. The frequency of sEPSCs in SGCs from sham-injured rats was significantly greater than in granule cells (sEPSC in Hz, GC median=1.65, IQR =0.88-3.68, n=3; SGC median=2.99, IQR=1.4-7.3, n=7). Molecular layer interneurons showed fewer spontaneous inhibitory inputs and a post-FPI increase in sIPSC frequency, indicating that location may not account for the differences in synaptic inputs between SGCs and granule cells. Morphometric analysis revealed a greater dendritic contraction angle in SGCs (in degrees, GC=59.1 ± 6.8, n=4; SGC=119.7 ±8.1, n=6, $p<0.05$, t-test). However, the total dendritic length was not different between the two cell types (in μm, GC=3206.9±377.4, n=5; SGC=2583.2±249.6, n=5). SGCs had more numerous first and second order branches and greater dendritic length in these low order, proximal branches than granule cells (dendritic length of first order branches in μm, GC=32.2±15.4, n=5; SGC=396.2±148.9, n=5; $p<0.05$, t-test). However, granule cells had greater dendritic length than SGCs at locations distal to the somata. Detailed morphological simulations of granule cells and SGCs incorporating identical active and passive properties suggest that the difference in morphology cannot fully explain the distinctive intrinsic physiology of SGCs.
Conclusions: These data reveal unique dendritic morphological characteristics of granule cells and SGCs that may contribute to the differences in their synaptic inputs and post-traumatic plasticity. However, our simulation studies indicate that, apart from the distinctive morphology, dissimilar channel distribution is likely to underlie differences in active properties between the cell types.
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1.007

TOLL-LIKE RECEPTOR 4 CONTRIBUTES TO EARLY INCREASE IN DENTATE EXCITABILITY AFTER CONCUSSIVE BRAIN INJURY BY NMDA RECEPTOR INDEPENDENT MECHANISMS

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Rationale: Traumatic brain injury, a leading cause of acquired temporal lobe epilepsy, results in cellular and synaptic changes including dentate cell loss and hyperexcitability. Release of endogenous molecules from disrupted cells and extracellular matrix can activate toll-like receptors (TLRs) involved in innate immune responses. Brain injury results in an increase in TLR4 expression within 4 hours followed by a return to control levels 7 days post-injury. Since TLR4 signaling has been implicated in hippocampal hyperexcitability in epilepsy, we examined whether increases in TLR4 expression contribute to early increases in dentate excitability after brain injury.
Methods: Young adult male Wistar rats (25-27 day old) were subject to sham- or moderate (2 atm) lateral fluid percussion injury (FPI) (Gupta et al., 2012). Immunostaining was used to determine the cell-type specific expression of TLR4. Afferent-evoked granule cell field responses were recorded in acute hippocampal slices prepared 3-7 days after FPI. Whole-cell current and voltage clamp recordings were obtained from granule cells and dentate hilar neurons in slices incubated in control ACSF or in LPS-RS for 2 hours.
Results: Expression of TLR4 in the dentate molecular layer and hilus was increased 24 hours after FPI. Hilar expression of TLR4 co-localized primarily with NeuN-positive neurons in both control and injured rats. Hilar neuronal profiles expressing somatostatin but not those expressing parvalbumin showed co-labeling for TLR4 indicating cell-type specific expression of TLR4 in GABAergic interneurons. LPS-RS decreased the amplitude of the perforant path-evoked granule cell population spike amplitude (amplitude in mV evoked by a 4mA stimulation, ACSF: 1.29 ± 0.27, LPS-RS: 0.38 ± 0.11 mA, $p<0.05$, n=15) in slices from FPI rats but not in sham-controls. The ability of LPS-RS to reduce the granule cell population spike amplitude in slices from injured rats was not altered in the presence of the selective NMDA receptor antagonist AP-5. In whole cell recordings, incubation in LPS-RS reduced the frequency and amplitude of spontaneous EPSCs and reduced firing in response to current injections in granule cells from injured rats. The ability of LPS-RS to reduce the post-FPI increase in granule cell population spike amplitude was decreased in the presence of the GABAA receptor antagonist SR95531 (10 μM) indicating that TLR4 signaling modifies dentate inhibition. The frequency and amplitude of sEPSCs in dentate interneurons was increased after FPI. Similar to granule cells, LPS-RS significantly reduced sEPSC frequency in dentate interneurons after FPI.
Conclusions: These findings demonstrate that TLR4 signaling contributes to early enhancement of dentate excitability after brain injury through NMDAR-independent mechanisms that likely involve complex cell-type specific modulation of excitatory and inhibitory circuits.
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1.008

THE WNT SIGNALING PATHWAY IS ACTIVATED DURING STATUS EPILEPTICUS AND EPILEPTOGENESIS

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Rationale: Wnt signaling has significant roles in brain development, neuron growth and guidance. Wnt signaling is active in specific regions of the adult brain, including the hippocampus, cortex and other regions. Wnt signaling is required for maintenance of neuronal stem cells. In status epilepticus (SE) and the post-SE epileptogenic period, there is significant expansion of resident stem cells and neuronal remodeling. We reasoned that Wnt signaling might be altered in the epileptogenic period and then investigated Wnt signaling activity in multiple experimental models to establish general principles. We conclude that Wnt signaling is robustly activated in the post-SE, epileptogenic period.
Methods: 7-week-old mice of appropriate background strains were treated with kainate (KA)/(C57bl6) or pilocarpine (PILO)/(FVBN). The brain regions were dissected between 1 hr. and 7 days following SE. Wnt signaling pathway activity was measured by a combination of qRT-PCR and quantitative western blots. Statistical significance was assessed with Instat software. In vivo KA-induced Wnt activation in forebrain neurons was assessed using the Wnt pathway reporter transgenic mouse line BAT-GAL (in C57bl6).
Results: Using the KA/C57bl6 mouse model, Wnt signaling was activated in the hippocampus rapidly and transiently with SE (1-2 hr.) then maximally at 5 days in the post-SE epileptogenic period. At day 5, we observed maximal induction of β-catenin levels, of the Wnt-target gene MYC, and of MYC targets LDHA and PK-M2. A detailed qRT-PCR analysis with a Wnt pathway specific gene array revealed that 23 known Wnt target genes were significantly induced, including LEF1, TCF4 and TCF7. In vivo analysis of Wnt signaling in BAT-GAL mice corroborated the biochemical analyses and further refined the location of highest Wnt signaling to the dentate gyrus and CA1 region. Using the PILO/FVBN mouse model, a similar analysis showed identical Wnt signaling and target gene activation patterns. Lastly, similar results were additionally obtained in equivalent rat SE models. Together, these data underscore that activated Wnt signaling is a general feature of SE and of epileptogenesis.
Conclusions: Molecular and in vivo analyses demonstrate that Wnt signaling is induced in the hippocampus in the post-SE epileptogenic period in multiple models. Furthermore, the induction of Wnt signaling in the dentate gyrus suggests that Wnt signaling may play a role in the neuronal reorganization that occurs in the epileptogenic period. These results establish Wnt signaling as a general feature of epileptogenesis in multiple experimental models. Our findings further suggest that the attenuation of Wnt signaling (by numerous drugs under development for cancer) may have unexpected efficacy for disease modification in the complex process of epileptogenesis. Elaboration of a molecular framework for investigating Wnt signaling should expedite the testing of new therapeutic strategies for epilepsy.
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1.009

MONOSYNAPTIC INPUTS TO NEONATALLY- VERSUS ADULT-BORN DENTATE GRANULE CELLS IN A RODENT MODEL OF TEMPORAL LOBE EPILEPSY

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Rationale: Neurogenesis persists throughout adulthood in the hippocampal dentate gyrus (DG) and is stimulated by seizures. In rodent temporal lobe epilepsy (TLE) models, dentate granule cells (DGCs) born after status epilepticus (SE) develop aberrant connections and may contribute to pathophysiology. To examine how