Published in final edited form as:

Acta Neurol Scand. 2012 January ; 125(1): 30–37. doi:10.1111/j.1600-0404.2011.01521.x.

Isolated Seizures in Rats do not Cause Neuronal Injury

Maria T. Acosta^{1,2}, Jeeva Munashinge³, Luhua Zhang³, Alfredo Guerron^{1,3}, Alexander Vortmeyer^{4,*}, and William H Theodore²

¹ Department of Neurology, Children's National Medical Center, Washington, DC, USA

² Clinical Epilepsy Section

³ Mouse Imaging Facility

⁴ Surgical Neurology Branch, National Institute of Neurological Disease and Stroke, NIH, Bethesda, MD, USA

Abstract

Background—Previous studies have shown that status epilepticus can lead to neuronal injury. However, the effect of a small number of isolated seizures is uncertain.

Methods—We used structural MRI and neuropathology to study the effects of isolated seizures induced by KA, ATPA and AMPA in rats. A group of animals received normal saline. After seizure induction, animals were followed for 12 weeks.

Results—ATPA and KA led to small but significant increases in ADC. There were no changes in T2 signal intensity or hippocampal volume. Blinded pathological examination showed no differences between animals receiving saline or glutamatergic agents.

Conclusion—Our study suggests that isolated seizures cause minimal neuronal injury in rats.

Neuronal injury due to prolonged seizures or status epilepticus occurs in several animal epilepsy models. Intrahippocampal (1) or intraperitoneal (2) Kainic Acid (KA) injection led to increased T2 signal on MRI, as did seizures in the lithium-pilocarpine model (3). Pilocarpine-induced status led to hippocampal volume loss and impaired Morris Water Maze performance (4).

In contrast, after amygdalar kindling without an initial episode of status epilepticus, neuronal loss was not found in CA1, CA3 or amygdala (5). Significant reductions in neuronal density were found in the dentate hilus, but these did not differ between rats with and without spontaneous seizures.

In a previous study, we found that intra-amygdalar injection of 10 nm KA, AMPA or ATPA GluR agonists evoked bilateral seizure activity and increased rCBF (6). In this study, we sought to determine whether isolated seizures, whether single or repetitive, induced by threshold doses of excitatory amino acids (EAAs), and not leading to status epilepticus, would lead to structural and pathological changes in rats. Studies in the kindling model suggested that AMPA receptors were critical for seizure expression, but less important for

Address Correspondence to: William H Theodore MD National Institutes of Health Building 10 Room 5N-250 Bethesda MD 20892 USA Phone: 301-496-1505 FAX: 301-402-2871 theodorw@ninds.nih.gov .

*present address: Department of Neurosurgery, Yale University School of Medicine, New Haven CT USA

Disclosures: None of the authors has any conflict of interest to disclose.

All authors performed the work reported as part of their official US Government duties.

epileptogenesis (7). Thus, we hypothesized that ATPA and KA were more likely than AMPA to cause chronic MRI changes reflecting development of an epileptogenic lesion.

Methods

Male Sprague-Dawley rats (Taconic Farms Inc) weighing 200 grams had three platinum MRI compatible cortical surface electrodes implanted, on the cortical surface of the right and left hemispheres as well as a reference over the cerebellum under isoflurane anesthesia. After 10 days rest, baseline MRI scan was performed, followed by tail vein cannulation for drug administration. Four animals each received ATPA((RS-2-amino-3-(3-hydroxy-5-tert-butylisoxazole-4-yl)propanoic acid) , AMPA(α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) , KA, or saline, at a concentration of 0.025 mmol/ml, and a rate of 0.125 ml/min. The infusion was continued until clinical or EEG evidence of seizure activity occurred, or a maximum of 2 ml was infused. After EEG recording for 151 ± 36 minutes, animals were returned to holding cages and an additional two to four hours of clinical observation carried out. All were given one dose of 5 milligrams per kilogram valium either at the end of the observation period, or as soon as any clinical or electrographic seizure activity was noted.

Subsequently, animals had weekly EEG and behavioral monitoring in sessions lasting two to four hours. EEG was observed visually for epileptiform discharges and high voltage slow waves. Subsequent MRIs were performed 24-72 hours after drug administration, and repeated at 2, 4, and 12 weeks. No additional convulsant drugs were given.

MRI

Rats were anesthetized with 1.5% isoflurane, intubated, paralyzed with pancuronium, placed in a stereotaxic holder and mounted in a 72/25 mm, transmit/receive coil ensemble. Body core temperature was maintained at 37° C using a circulating water pad and monitored with a rectal temperature probe. A tail vein line was placed for drug infusion. MRI was performed on a horizontal 7 Tesla (Bruker Avance, Billerica, MA), 21 cm horizontal scanner.

Fast spin echo (FSE) images of three mutually perpendicular slices were acquired through the brain as scout images. Using these scout images, eight 1 mm thick, amygdale centered, axial slices were acquired using a FSE sequence for regional anatomical delineation (TE/TR = 10/2000 ms, echo train length=8, In-plane resolution = 125 μ m). Quantitative T₂ weighted (TE = 10 ms, 16 echoes, TR = 3000 ms, Matrix 128², In-plane resolution = 250 μ m) and diffusion weighted (in three orthogonal directions) sequences (b = 4, from 0 to 3000 Gauss/cm, TE/TR = 20/2500 ms, Δ = 32 ms, matrix = 128²), with same geometry (FOV = 3.2 cm) as qualitative scans, were performed. These measurements were repeated at each time point, pre-drug, 24 hrs post drug, 1 week, 2 week, 4 weeks and 12 weeks.

T₂ and ADC maps, where each image pixel depicted the respective quantitative entity, were generated using routines developed in MATLAB (Mathworks, Inc., Natick, MA). Quantitative T₂ and ADC values were calculated for regions of Interest (ROI) placed in left and right amygdala, hippocampus, thalamic region, sensory and motor cortices of both hemispheres to investigate any widespread brain activation.

Data from each individual ROI were averaged across slices (4-8). Furthermore, in order to avoid the inter-individual rat variability, all data were then normalized to each animal's baseline, pre-infusion MRI results on a region by region basis. Data analysis, including effects of drug on MRI parameters and seizure activity, and the relation of initial seizure score to MRI findings, was performed with analysis of variance and post-hoc Bonferroni's Test with Systat (Systat Inc. Richmond CA).

EEG

Bipolar recordings were performed using AcqKnowledge™ 3.7 software, (Biopac Systems, Inc, CA, USA.). EEG was interpreted by two investigators with previous experience in rat EEG.

For the purposes of our study, seizures were defined clinically. Epileptiform discharges occurring without clinical manifestations were considered to be interictal. In order to examine the relation between clinical and electrographic seizure activity and changes in MRI parameters, we used the following scoring system:

- 0: No EEG or clinical effects
- 1. EEG epileptiform discharges but no clinical seizures; normal behavior maintained.
- 2. Minor motor activity, such as freezing, mouth movement, twitching
- 3. More pronounced behavioral changes, such as head nodding, forelimb clonus, running
- 4. Generalized seizures with falling.

Pathology

After the last MRI, animals were anesthetized with isoflurane, and perfused with saline and 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde, followed by intracardiac KCL.

The brains were removed and postfixed in the same fixative for 6-9 hours at 4°C. Following immersion in 0.1 M phosphate-buffered saline (PBS; pH 7.4) containing 20% sucrose for 48 hours at 4°C, the brains were rapidly frozen in isopentane pre-cooled to -70°C with dry ice and stored at -75°C.

Serial cryostat sections (50 & 30 µm) were cut coronally through the whole brain including the cerebellum and brain stem, approximately from bregma 4.70 mm to bregma -14.60 mm (cf. the Rat Brain in Stereotaxic Coordinates by Paxinos & Watson, 1986). Every 1st (50 µm), 2nd (50 µm), 3rd (50 µm), 4th (50 µm), 5th (30 µm), 6th (30 µm), 7th (30 µm) and 8th (30 µm) sections of each series of 8 sections were collected separately. The sections of the 3rd set (50 µm) were mounted on 1"×3" Superfrost Plus microscope slides (3 sections per slide) and stained with cresyl violet.

The sections of the 4th set (50 µm) were collected in 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde and stored at 4°C for 5 days. Sections were then processed for the detection of neurodegeneration with FD Neurosilver™ Kit II (FD Neurotechnologies, Inc, Baltimore, MD; www.fdneurotech.com). Subsequently, all sections were mounted on slides, dehydrated in ethanol, cleared in xylene, and coverslipped with Permount® (Fisher Scientific, Fair Lawn, NJ).

The sections of the 5th set (30 µm) were processed for OX42 immunohistochemistry (8). Thus, after inactivating the endogenous peroxidase activity with hydrogen peroxidase, sections were incubated free-floating in 0.01 M PBS containing 1% normal horse serum (Vector Lab., Burlingame, CA), 0.3% Triton X-100 (Sigma, St. Louis, MO) and a monoclonal mouse anti-OX42 IgG (1: 20,000; Accurate, Westbury, NY) for 3 days at 4°C. The immunoreaction product was then visualized according to the avidin-biotin complex method of Hsu et al. (1981) with the Vectastin elite ABC kit (Vector Lab., Burlingame, CA). Briefly, sections were incubated in PBS containing biotinylated horse anti-mouse IgG, Triton-X and normal horse serum for 1 hour and then in the PBS containing avidin-

biotinylated horseradish peroxidase complex for another hour. This was followed by incubation of the sections for 10 minutes in 0.05 M Tris buffer (pH 7.2) containing 0.03% 3',3'-diaminobenzidine (Sigma) and 0.0075% H₂O₂. All steps were carried out at room temperature except indicated, and each step was followed by washes in PBS. After thorough rinses in distilled water, all sections were mounted on slides, dehydrated in ethanol, cleared in xylene, and coverslipped in Permount®.

Results

Rats received 24.1 ± 1.5 mg/kg of AMPA, 16.7 ± 2.6 mg/kg ATPA, and 21.5 ± 4.7 mg/kg KA. During drug infusion, four animals had level 1 activity, two had level 2, five had level 3, and 1 (receiving AMPA) had level 4. Valium was given to animals when seizure activity began; none lasted longer than 5 minutes. The effect of the valium dose was monitored both clinically and by EEG, since the goal was to minimize initial seizure activity. During the subsequent 12 week observation, no animal had greater than level 1 activity (EEG discharges alone, without clinical manifestations). No animal receiving saline showed any EEG or clinical effects.

EEG

Ictal electrographic activity was present during drug infusion in the context of minimal clinical seizure activity; no animal developed SE at any time, and intermittent ictal and interictal EEG discharges persisted during the observation period for all convulsants.

T2

On the immediate post-infusion scan, there was a non-significant trend for a drug effect (F-ratio 2.56; $p < 0.10$) with AMPA slightly lower than other drugs (table 1). However, no effects of drug group were found on subsequent scans. Similar results were found for absolute T2 values. There was no significant effect of region on T2 at any time point (figure 1).

ADC

There were significant effects of drug on normalized ADC values on the immediate post infusion (F-ratio 7.547; $p < 0.001$), week 1 (F-ratio 12.946, $P < 0.001$), week 2 (F-13.885; $p < 0.001$), week 4 (F-ratio 8.014, $p < 0.001$) and week 12 scans (F-ratio 5.671 $p < 0.002$). Values for ATPA and KA increased, while AMPA and saline tended to show relative decreases (figure 2). At 12 weeks, KA values were significantly higher than AMPA, but the latter was not different from saline or ATPA.. There were no significant regional variations.

Hippocampal Volume

There were no significant differences in hippocampal volume attributable to drug over the 12 week observation period (figure 3).

Relation between seizure activity and MRI data

ATPA led to significantly higher initial, and AMPA late seizure scores than the other drugs ($P < 0.001$). Across all regions, there was no relation between seizures occurring either at the time of infusion or during the 12 week observation and changes in T2 (F-ratio 0.82). However, when amygdala and hippocampus were analyzed alone, there was a significant positive relation of both seizure score during infusion (F-ratio 3.8; $P < 0.01$) to normalized week 12 T2 signal. There was a non-significant trend (F-ratio 2.4; $P < 0.07$) for an association between seizure score during infusion and T2 immediately after infusion.

Over all regions, or in amygdala and hippocampus alone, there were no significant effects of seizures during infusion, or the observation period, on week 12 normalized ADC.

Pathology

Several animals showed hemosiderin deposition and superficial cortical traumatic changes consistent with electrode placement (figure 4). There were no pathologic changes in hippocampus in rats receiving convulsants who had seizures, compared with saline controls (figure 5)

Discussion

In our study, rats exposed to three excitatory amino acid convulsant drugs experienced varying levels of clinical seizure activity during initial administration, but none had status epilepticus. Subsequently, we observed no further clinical seizures. Serial MRI showed no overall effects on T2 signal intensity over 12 weeks, but higher seizure scores did correlate with increased T2. ATPA and KA led to small increases in ADC, but there was no independent effect of seizure score. No pathological changes were observed in any animal, aside from those due to electrode placement. Histologic analysis alone may not be sensitive enough to detect minimal structural deviations. In this study, cresyl violet stains were complemented with silver stains for better visualization of axons. In addition, OX42 immunohistochemistry was routinely performed allowing for sensitive identification of microglial/macrophagocytic responses to minor injuries.

Previous studies of the effect of isolated seizures have shown conflicting results. Quantitative stereology showed neuronal loss in dentate hilus and CA1 after three generalized tonic-clonic seizures, reaching 49% and 44% of controls after 150 seizures (9). Neuronal loss was detected in CA3, entorhinal cortex, and rostral endopyriform nucleus after 30 seizures, and the granule cell layer and CA2, but not somatosensory cortex after 150 seizures. A brief non-convulsive seizure, lasting less than two minutes, evoked by electrical kindling led to bilateral dentate apoptosis identified by TUNEL stain (10). Apoptotic cell death, identified by ApopTag-positivity, in the hippocampus increased 30.4% after one kindled seizure and 82.5% after 20 seizures compared to sham controls (11).

In contrast, six months after induction of amygdala kindling, there was no decrease in the total number of neurons in amygdala or hilus in kindled compared to sham-operated rats, and was no correlation between total afterdischarge duration or number of electrical stimulations and neuronal number counted with stereology (12). The animals in this study had approximately five secondarily generalized and five milder seizures. Another study found no difference in hippocampal damage between rats that did and did not have spontaneous seizures after long-term hippocampal stimulation (5). The dentate cell loss observed could have been due to the kindling itself. Pharmacologic protection after 90 minutes of KA-induced status reduced limbic apoptotic injury, but not recurrent spontaneous seizures (13).

MRI evidence for effects of single or multiple seizures without status is limited. One study showed increased T2 signal two weeks after rat amygdala-kindled seizures without status epilepticus, but no difference in hippocampal volumes (14).

The value of MRI for prediction of neuronal injury in experimental seizure models is uncertain. Broadly parallel changes in MRI and pathology were found in rats developing spontaneous seizures after lithium-pilocarpine status (3). In immature rats with prolonged febrile seizures, abnormal T2 signal in dorsal hippocampus, piriform cortex, and amygdala was not associated with Fluoro-Jade evidence of neuronal injury or death (15). Even in

status models MRI changes may be transient or fluctuating. T2, T1 ρ , and Dav values increased in amygdala, piriform cortex, midline thalamus, and hippocampus two days after status induced by amygdala kindling (16). These signal changes receded by nine days, but new abnormalities appeared at 20 days, when spontaneous seizures occurred. However, initial MRI findings were not predictive of either seizures or tissue damage.

A recent study compared rats given pilocarpine that did and did not develop initial status with saline controls; animals without initial status developed spontaneous seizures after a mean of 8 months (17). Only animals who had initial status had increased T2 signal at 24 hours; at one year, however, animals without initial status had increased T2 compared to both other groups. Cerebral blood volume, however, had been elevated in both pilocarpine groups compared with control at 24 hours. The results are interesting in comparison to our findings, because we detected small but significant early changes in CBF but not ADC in animals after mild seizures.

We saw differential effects of KA and to a lesser degree ATPA compared to AMPA on ADC. The most likely explanation is the absence of the GluR5 receptor subunit in the AMPA complex, and the higher pharmacologic activity of KA (18-19). Delayed changes in ADC, with regional variation of increases and decreases probably related to differing sensitivity to cytotoxic and vasogenic edema, have been reported in status epileptic models (20-21). One study suggested that increased ADC in chronic lesions such as epileptic foci (as opposed to acute ischemia) may be due to an expansion of the extracellular space, with structural preservation (22-23). The observation may best be explained by the concept that expansion in the extracellular space, which causes increased diffusivity, is probably associated with a relatively of the fiber bundle. It is possible that this reflects progression of underlying tissue injury, and in our study may suggest subtle tissue physiologic disturbance (24).

Seizures can lead to functional impairment without permanent neuronal injury. In rats, daily flurothyl-induced generalized seizures led to reversible decline in a spatial accuracy task, followed by return to baseline performance once seizures stop. (25). Immature rats exposed to febrile seizures have lowered convulsant thresholds without spontaneous seizures, suggesting enhanced excitability (26).

Rats with elevated hippocampal T2 on MRI after febrile status had significantly worse Learning and memory than those of T2-negative animals or controls (27). However, there were no significant differences in hippocampal cell counts.

In clinical studies, prolonged febrile seizures have been associated with acute hippocampal T2 signal increases, suggesting tissue injury (28). Patients with persistent uncontrolled temporal lobe epilepsy have reduced hippocampal volume that may be related to epilepsy duration or seizure frequency, particularly for generalized tonic-clonic seizures (29-30). Most of the patient reported in these studies have had frequent seizures for many years, although not all have well-documented episodes of status epilepticus.

The clinical effect of isolated or infrequent seizures is uncertain. In patients with newly diagnosed epilepsy, there was no overall difference in hippocampal volumes at baseline or on repeat scan at five years (31). A small proportion that showed decreased hippocampal volume had longer epilepsy duration and more seizures before the baseline scan, but not during the interscan interval, than patients who did not show volume loss. In a study of patients with 'mild' temporal lobe epilepsy over 3.5 years, hippocampal volume loss was correlated with the number of generalized tonic-clonic seizures between MRI scans (32). Seizure-induced structural damage was not detected by MRI repeated at 3.5 years after newly-diagnosed epilepsy in an outpatient population (33). Although only 56% of the

patients had more than a single seizure, the data suggest that rare seizures are unlikely to cause neuronal injury detectable by clinical imaging. Although technique-dependent to some extent, human MRI hippocampal T2 signal and volumetry do reflect pathological changes.

Several other MRI-based techniques have potential applications in studying small animal seizure models. Magnesium chloride-enhanced MRI may show regions of neuronal activation associated with altered uptake and transport (34). Increased dentate gyrus and CA3 Mn(2+)-enhanced pixels after KA injection in rats correlated with mossy fiber sprouting (35). Diffusion tensor imaging has potential clinical as well as research application, suggestion possible fiber tract reorganization, but clinical correlation has been limited. (34, 36). Following SE, 8/11 injured rats developed spontaneous seizures. T2, diffusivity and anisotropy changes occurred early in parahippocampal gyrus contralateral to electrical stimulation inducing status epilepticus in rats, as well as in bilateral fimbria (37). When excised brains were scanned at 60 days, bilateral increased anisotropy was found in dentate, where Timm staining showed mossy fiber sprouting. New sequences such as 'Propeller' and 'STIR' may improve hippocampal visualization (36).

Our study has several limitations, and our conclusions must be tentative. All the animals received a dose of diazepam after the initial EEA infusion. This may have had a therapeutic effect. The period of study before sacrifice may not have been long enough to allow pathological changes to develop. The number of rats we could study was low. It is possible that failure to include groups of animal sacrificed at earlier time points led us to miss transient neuropathological changes, and that a quantitative morphologic method might have been more sensitive. Unfortunately, we did not have access to continuous video-EEG monitoring equipment for the study, and could only carry out limited periods of observation over the 12 weeks after seizure induction. However, underestimation of the number of clinical seizures and epileptiform discharges would not vitiate our suggestion that isolated seizures induced by glutamatergic agonists leads to subtle changes in t2 signal and ADC, but not neuropathologic damage in rats.

Acknowledgments

Supported by the NIH NINDS Division of Intramural Research.

References

1. Bouilleret V, Nehlig A, Marescaux C, Namer LJ. Magnetic resonance imaging follow-up of progressive hippocampal damage in a mouse model of mesial temporal lobe epilepsy. *Epilepsia*. 2000; 41:642–50. [PubMed: 10840394]
2. Wolf OT, Dyakin V, Patel A, Vadasz C, de Leon MJ, McEwen BS, Bulloch K. Volumetric structural magnetic resonance imaging (MRI) of the rat hippocampus following kainic acid (KA) treatment. *Brain Res*. 2002; 934:87–96. [PubMed: 11955471]
3. Roch C, Leroy C, Nehlig A, Namer IJ. Magnetic resonance imaging in the study of the lithium-pilocarpine model of temporal lobe epilepsy in adult rats. *Epilepsia*. 2002; 43:325–35. [PubMed: 11952761]
4. Niessen HG, Angenstein F, Vielhaber S, Frisch C, Kudin A, Elger CE, Heinze H-J, Scheich H, Kunz WS. Volumetric Magnetic Resonance Imaging of Functionally Relevant Structural Alterations in Chronic Epilepsy after Pilocarpine-induced Status Epilepticus in Rats. *Epilepsia*. 2005; 46:1021–1026. [PubMed: 16026554]
5. Brandt C, Ebert U, Loscher W. Epilepsy induced by extended amygdala-kindling in rats: lack of clear association between development of spontaneous seizures and neuronal damage. *Epilepsy Research*. 2004; 62:135–156. [PubMed: 15579302]

6. Munasinghe JP, Banerjee M, Acosta MT, Banks M, Heffer A, Silva AC, Koretsky A, Theodore WH. Arterial spin labeling demonstrates that focal amygdalar glutamatergic agonist infusion leads to rapid diffuse cerebral activation. *Acta Neurol Scand.* 2010; 121:209–216. [PubMed: 19951270]
7. Rogawski MA, Kurzman PS, Yamaguchi SI, Li H. Role of AMPA and GluR5 kainate receptors in the development and expression of amygdala kindling in the mouse. *Neuropharmacology.* 2001; 40:28–35. [PubMed: 11077068]
8. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* 1981; 29:577–580. [PubMed: 6166661]
9. Cavazos JE, Das I, Sutula TP. Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. *J Neurosci.* 1994; 14:3106–3121. [PubMed: 8182460]
10. Bengzon J, Kokaia L, Elmer E, Nanohashvilli A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc Natl Acad Sci USA.* 1997; 94:10432–10437. [PubMed: 9294228]
11. Zhang L-X, Smith MA, Li X-L, Weiss SRB, Post RM. Apoptosis of hippocampal neurons after amygdala kindled seizures. *Molecular Brain Research.* 1998; 55:198–208. [PubMed: 9582422]
12. Tuunanen J, Pitkanen A. Do seizures cause neuronal damage in rat amygdala kindling? *Epilepsy Res.* 2000; 39:171–176. [PubMed: 10759304]
13. Ebert U, Brandt C, Loscher W. Delayed sclerosis, neuroprotection, and limbic epileptogenesis after status epilepticus in the rat. *Epilepsia.* 2002; 43(suppl 5):86–95. [PubMed: 12121301]
14. Jupp B, Williams JP, Tesiram YA, Vosmansky M, O'Brien T. Hippocampal T2 Signal Change during Amygdala Kindling Epileptogenesis. *Epilepsia.* 2006; 47:41–46. 2006. [PubMed: 16417530]
15. Dubé C, Yu H, Nalcioğlu O, Baram TZ. Serial MRI after experimental febrile seizures: altered T2 signal without neuronal death. *Ann Neurol.* 2004; 56:709–14. [PubMed: 15389889]
16. Nairismagi J, Grohn OHJ, Kettunen MI, Nissinen J, Kauppinen RA, Pitkanen A. Progression of Brain Damage after Status Epilepticus and Its Association with Epileptogenesis: A Quantitative MRI Study in a Rat Model of Temporal Lobe Epilepsy. *Epilepsia.* 2004; 45:1024–1034. [PubMed: 15329065]
17. Mora GN, Bramanti P, Osculati F, Chakir A, Marzola EP, Sbarbati A, Fabene PF. Does Pilocarpine-Induced Epilepsy in Adult Rats Require Status epilepticus? *PloS ONE.* 2009; 4:e5759. [PubMed: 19503612]
18. Meldrum BS, Rogawski MA. Molecular Targets for Antiepileptic Drug Development. *NeuroTherapeutics.* 2007; 4:18–61. [PubMed: 17199015]
19. Kessels HW, Malinow R. Synaptic AMPA Receptor Plasticity and Behavior. *Neuron.* 2009; 61:340–350. [PubMed: 19217372]
20. Cox JE, Mathews VP, Santos CC, Elster AD. Seizure-induced transient hippocampal abnormalities on MR: correlation with positron emission tomography and electroencephalography. *AJNR Am J Neuroradiol.* 1995; 16:1736–1738. [PubMed: 7502985]
21. Wall CJ, Kendall EJ, Obenaus A. Rapid alterations in diffusion-weighted images with anatomic correlates in a rodent model of status epilepticus. *AJNR Am J Neuroradiol.* 2000; 21:1841–52. [PubMed: 11110536]
22. Wieshmann UC, Clark CA, Symms MR, Barker GJ, Birnie KD, Shorvon SD. Water diffusion in the human hippocampus in epilepsy. *Magn Reson Imaging.* 1999; 17:29–36. [PubMed: 9888396]
23. Assaf, Bassam A.; Mohamed, Feroze B.; Abou-Khaled, Karine J.; Williams, J. Michael; Yazeji, May S.; Haselgrove, John; Faro, Scott H. Diffusion Tensor Imaging of the Hippocampal Formation in Temporal Lobe Epilepsy. *AJNR Am J Neuroradiol.* 2003; 24:1857–1862. [PubMed: 14561616]
24. Grohn O, Pitkainen A. Magnetic Resonance Imaging in Animal Models of Epilepsy—Noninvasive Detection of Structural Alterations. *Epilepsia.* 2007; 48(Suppl. 4):3–10. [PubMed: 17767570]
25. Lin H, Holmes GL, Kubie JL, Muller RU. Recurrent Seizures Induce a Reversible Impairment in a Spatial Hidden Goal Task. *Hippocampus.* 2009; 19:817–27. (2009). [PubMed: 19235227]

26. Dube C, Chen K, Eghbal-Ahmadi M, Brunson K, Soltesz I, Baram TZ. Prolonged Febrile Seizures in the Immature Rat Model Enhance Hippocampal Excitability Long Term. *Ann Neurol.* 2000; 47:336–344. [PubMed: 10716253]
27. Dubé CM, Zhou JL, Hamamura M, Zhao Q, Ring A, Abrahams J, McIntyre K, Nalcioğlu O, Shatskih T, Baram TZ, Holmes GL. Cognitive dysfunction after experimental febrile seizures. *Exp Neurol.* 2009; 215:167–77. [PubMed: 19000675]
28. Van Landingham KE, Heinz ER, Cavazos JE, Lewis DV. Magnetic resonance imaging evidence of hippocampal injury after prolonged focal febrile convulsions. *Ann Neurol.* 1998; 43:413–26. [PubMed: 9546321]
29. Kalviainen R, Salmenpera T, Partanen K, et al. Recurrent seizures may cause hippocampal damage in temporal lobe epilepsy. *Neurology.* 1998; 50:1377–1382. [PubMed: 9595990]
30. Theodore WH, Bhatia S, Hatta J, Fazilat S, DeCarli C, Bookheimer S, Gaillard WD. Hippocampal Atrophy, Epilepsy Duration, and Febrile Seizures in Patients with Partial Seizures. *Neurology.* 1999; 52:132–6. [PubMed: 9921860]
31. Salmenperä T, Könönen M, Roberts N, Vanninen R, Pitkänen A, Kälviäinen R. Hippocampal damage in newly diagnosed focal epilepsy: a prospective MRI study. *Neurology.* 2005; 64:62–8. [PubMed: 15642905]
32. Briellmann RS, Berkovic SF, Syngieniotis A, King MA, Jackson GD. Seizure-associated hippocampal volume loss: a longitudinal magnetic resonance study of temporal lobe epilepsy. *Ann Neurol.* 2002; 51:641–4. [PubMed: 12112114]
33. Liu RSN, Lemieux L, Bell GS, et al. The Structural Consequences of Newly Diagnosed Seizures. *Ann Neurol.* 2002; 52:573–580. [PubMed: 12402254]
34. Obenaus A, Jacobs RE. Magnetic Resonance Imaging of Functional Anatomy: Use for Small Animal Epilepsy Models. *Epilepsia.* 2007; 48(Suppl. 4):11–17. [PubMed: 17767571]
35. Nairismagi J, Pitkanen A, Narkilahti S, Huttunen J, Kauppinen RA, Grohn OH. Manganese-enhanced magnetic resonance imaging of mossy fiber plasticity in vivo. *Neuroimage.* 2006; 30:130–135. [PubMed: 16246593]
36. Duncan J. The current status of neuroimaging for epilepsy. *Curr Opin Neurol.* 2009; 22:179–84. [PubMed: 19300096]
37. Parekh MB, Carney PR, Sepulveda H, Norman W, King M, Mareci TH. Early MR diffusion and relaxation changes in the parahippocampal gyrus precede the onset of spontaneous seizures in an animal model of chronic limbic epilepsy. *Exp Neurol.* 2010; 224:258–70. [PubMed: 20394745]

Figure 1. Average T_2 variation of left and right hippocampi over 12 weeks in animals ($n=4$ each) exposed to saline, AMPA, KA, and ATPA. Although individual variations and an age related decrease in T_2 were apparent, there were no significant effects of drug on T_2 at any time point.

Figure 2.

Average Hippocampal ADC measurements over 12 weeks in animals (n=4 each) exposed to saline, AMPA, KA, and ATPA. Similar to the T2 variations, significant ADC variations were not observed over time for any drug.

Figure 3. Hippocampal volume measurements over 12 weeks in animals (n=4 each) exposed to saline, AMPA, KA, and ATPA. There were no significant differences in hippocampal volume attributable to drug over the 12 week observation period.

Figure 4.
OX 42 (A) and Nissl Stains (B) showing superficial cortical trauma due to electrode placement in an animal that received saline. 10 × magnification.

Figure 5. Transverse T₂ weighted MRI section (A) and hippocampal slice (C) (Nissl Stain 2.5 × magnification) from an animal that received saline. MRI section (B) and hippocampal slice (D) from an animal that received kainic acid and experienced level 3 seizure activity during drug infusion. There were no pathological or MRI effects of convulsants.

Table 1

mean normalized T2 values.

Drug	Post	Week 1	Week 2	Week 4	Week 12
AMPA	0.98 ± 0.03	0.98 ± 0.03	0.98 ± 0.04	0.97 ± 0.03	0.97 ± 0.05
ATPA	0.99 ± 0.05	0.98 ± 0.04	0.99 ± 0.04	0.99 ± 0.06	0.98 ± 0.05
KA	1.00 ± 0.03	1.00 ± 0.04	0.99 ± 0.04	0.99 ± 0.03	0.98 ± 0.04
Saline	0.99 ± 0.04	0.99 ± 0.04	0.99 ± 0.04	1.00 ± 0.05	0.97 ± 0.05

Table 2

mean Normalized ADC values

Drug	Post	Week 1	Week 2	Week 4	Week 12
AMPA	0.97 ± 0.12	0.99 ± 0.05	0.96 ± 0.06	1.00 ± 0.06	0.95 ± 0.08
ATPA	1.05 ± 0.09	1.04 ± 0.08	1.02 ± 0.06	0.95 ± 0.10	0.98 ± 0.06
KA	1.00 ± 0.09	1.04 ± 0.06	1.03 ± 0.08	1.03 ± 0.10	1.01 ± 0.09
Saline	0.99 ± 0.06	0.98 ± 0.08	0.97 ± 0.04	0.97 ± 0.04	0.96 ± 0.06