Published in final edited form as:

Epilepsia. 2007 August ; 48(8): 1470–1476. doi:10.1111/j.1528-1167.2007.01110.x.

THE NATURAL HISTORY AND TREATMENT OF EPILEPSY IN A MURINE MODEL OF TUBEROUS SCLEROSIS

Ebru Erbayat-Altay*, Ling-Hui Zeng*, Lin Xu, David H. Gutmann, and Michael Wong

Department of Neurology and the Hope Center for Neurological Disorders, Washington, University School of Medicine, St. Louis, MO 63110

Summary

Purpose—Patients with Tuberous Sclerosis Complex (TSC) often have severe epilepsy that is intractable to available therapies. The development of novel treatments for epilepsy in TSC would benefit greatly from a suitable animal model, but most animal models of TSC to date have few reported neurological abnormalities, such as epilepsy. We previously described a novel model of TSC, due to conditional inactivation of the *Tsc1* gene in glia (*Tsc1*^{GFAP}CKO mice), in which mice develop epilepsy and premature death. Here, we characterize the natural history of the epilepsy in *Tsc1*^{GFAP}CKO mice in more detail and report acute effects of treatment with standard antiepileptic drugs on seizures in these mice.

Methods—Video-EEG recordings were obtained from *Tsc1*^{GFAP}CKO mice on a weekly basis, starting at 4 weeks of age until death, to monitor progression of interictal EEG abnormalities and seizures. In separate experiments, *Tsc1*^{GFAP}CKO mice were monitored for interictal EEG abnormalities and seizures before and during treatment with phenobarbital, phenytoin, or saline.

Results—*Tsc1*^{GFAP}CKO mice developed seizures around 4–6 weeks of age and subsequently had progressive worsening of the interictal EEG background and seizure frequency over a month, culminating in death. Treatment with phenobarbital or phenytoin caused a reduction in seizure frequency, but did not improve EEG background or prevent death.

Conclusions—*Tsc1*^{GFAP}CKO mice develop progressive epilepsy. Acute treatment with standard antiepileptic drugs suppresses seizures in these mice, but does not affect long-term prognosis. *Tsc1*^{GFAP}CKO mice represent a good model to test other drugs that may have true anti-epileptogenic actions in TSC.

Keywords

Seizure; Antiepileptic Drug; Tuberous Sclerosis Complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is a relatively common genetic disease affecting multiple organ systems and caused by mutation of either the *TSC1* or the *TSC2* gene (Sparagana and Roach, 2000; Kwiatkowski, 2003; Crino et al., 2006). Neurological involvement in TSC usually produces the most disabling symptoms of the disease, including seizures, mental retardation, and autism. Epilepsy in TSC can be particularly severe, may involve multiple seizure types, including infantile spasms, and is often intractable to available medical and

Corresponding Author: Michael Wong, MD, PhD, Department of Neurology, Box 8111, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, Phone: 314-362-8713, Fax: 314-362-9462, wong_m@wustl.edu.

*These authors contributed equally to this work.

surgical treatments. Thus, novel therapies for epilepsy in TSC are clearly needed to improve the quality of life in TSC patients.

The development of new treatments for epilepsy in TSC would benefit greatly from a suitable small-animal model, but most animal models of TSC to date have few reported, overt neurological abnormalities, such as epilepsy. For example, the Eker rat, with a spontaneous mutation in the *Tsc2* gene, is probably the most widely used animal model of TSC. However, while some neuropathological abnormalities and alterations in neuronal plasticity are observed in this rat model of TSC (Mizuguchi et al., 2000; Wenzel et al., 2004; Takahashi et al., 2004; von der Brelie et al., 2006; Waltereit et al. 2006; Tschuluun et al., 2007), the Eker rat has not been documented to have an altered seizure threshold or spontaneous epilepsy (Waltereit et al. 2006; Tschuluun et al., 2007). Recently, we have described the neurological phenotype of a novel murine model of TSC (Uhlmann et al., 2002). Focusing on the prominent neuropathological abnormalities in glial cells seen in TSC patients (Mizuguchi and Takashima, 2001; Ess et al., 2004), this mouse model involves conditional inactivation of the *Tsc1* gene predominantly in glia. These *Tsc1*^{GFAP}CKO mice exhibit spontaneous seizures and premature death, as well as a number of cellular and molecular abnormalities in glia that may directly promote epileptogenesis in these mice (Uhlmann et al., 2002; Wong et al., 2003; Jansen et al., 2005). In the present study, we utilized video-EEG recording to characterize in more detail the natural history and progression of epilepsy, as well as background EEG abnormalities, in *Tsc1*^{GFAP}CKO mice – information critical for correlating potential cellular mechanisms of epileptogenesis with the temporal onset of seizures, as well as for establishing a baseline for preclinical drug trials. In addition, we report the results of initial drug trials of standard antiepileptic drugs on seizures in these mice, which may also serve as a comparative baseline for future studies of novel drugs, including those that may be able to prevent or reverse the underlying mechanisms of epileptogenesis in TSC.

MATERIALS AND METHODS

Animals and Materials

Care and use of animals conformed to a protocol approved by the Washington University School of Medicine Animal Studies Committee. *Tsc1*^{flox/flox}-GFAP-Cre knockout (*Tsc1*^{GFAP}CKO) mice with conditional inactivation of the *Tsc1* gene in glia were obtained from an existing breeding colony, originally created using Cre-LoxP technology as described previously (Uhlmann et al., 2002). All mice were maintained on a mixed 129sv genetic background. Drugs were obtained from Sigma (Saint Louis, MO).

Video-EEG monitoring and analysis

Epidural screw electrodes were surgically implanted under isoflurane anesthesia and secured using dental cement for long term video-EEG recordings. Four electrodes were placed on the skull: one right and one left central electrodes (1 mm lateral to midline, 2 mm posterior to bregma), one frontal electrode (0.5 mm anterior and 0.5 mm to the right or left of bregma) and one occipital electrode (0.5 mm posterior and 0.5 mm to the right or left lambda). The typical recording montage involved two EEG channels with the right and left central “active” electrodes being compared to either the frontal or occipital “reference” electrode. Previous studies also utilized hippocampal depth electrodes and detected rare seizures with initial onset in hippocampus, but all seizures secondarily generalized to bilateral neocortex (Uhlmann et al., 2002); thus, use of the simplified recording montage in the present study should be sufficient to obtain accurate seizure counts. Animals were allowed to recover from surgery for at least 24 hours before recording.

Video-EEG monitoring sessions always occurred in epochs lasting 48 hours. Continuous EEG data were saved digitally on personal computers using Grass P-100 AC amplifiers (Astro-Med, West Warwick, RI), Axon Digidata A-D converters, and Axoscope software (Molecular Devices, Sunnyvale, CA). To determine the behavioral correlate of electrographic seizures, simultaneous digital video was recorded using a Sanyo Day-Night camera and a Darim MG-100 MPEG video capture card (Darim Vision Corp., Pleasanton, CA).

For EEG analysis, every 48 hour epoch was analyzed for interictal EEG background activity and seizures. Analysis of the interictal EEG background occurred by a method similar to previous reported studies (Griffey et al., 2006; Kielar et al., 2007). For each 48 hour epoch, twelve one-minute samples were selected at standardized pre-set time points separated by exactly 4 hours. In the rare event when a seizure had occurred at or within ten minutes before the selected time, we used the one-minute sample that was 10 minutes after the end of the postictal phase. Each one-minute sample was assessed for the number of interictal epileptiform spikes present and a qualitative score of interictal background activity by a board-certified electroencephalographer who was blinded to the age and treatment of the mice. Interictal spikes were defined as fast (<200 ms) epileptiform waveforms that were at least twice the amplitude of the background activity. As described previously (Griffey et al., 2006; Kielar et al., 2007) and shown in Figure 1A, the scoring of the interictal background activity was based on a four grade scale: 1 - normal background activity (+/- 6–8 Hz sinusoidal theta rhythm), no epileptiform spikes; 2 - mostly normal background activity, few epileptiform spikes; 3 - mostly abnormal background activity, many spikes; 4 - burst-suppression pattern. An average score for spike frequency (spikes/minute) and background activity (grade 1–4) was calculated for each 48 hour epoch.

In addition to the interictal grading scale, a quantitative frequency analysis of the same interictal EEG samples was performed with pCLAMP software (Molecular Devices, Sunnyvale, CA), using Fast Fourier Transform with a Hamming window. Based on initial analysis of the data, a ratio of the power of theta (4–8 Hz) and delta (1–4 Hz) frequencies of the averaged interictal EEG data for each mouse at each age/treatment was calculated.

For seizure analysis, the entire EEG of each 48 hour epoch was reviewed for electrographic seizures. As previously documented (Uhlmann et al., 2002) and shown in Figure 2A, electrographic seizures in *Tsc1^{GFAP}*CKO mice are easily identified by a stereotypical pattern involving an initial onset of a tonic, repetitive spike discharge followed by a progressive evolution in spike amplitude and frequency that usually culminates in a bursting pattern and postictal suppression. The behavioral correlate of electrographic seizures was noted by corresponding time-linked video analysis. The average frequency (number of seizures/day) and duration of seizures was calculated for each 48-hour epoch.

Study Designs

The first set of experiments examined the natural history and progression of epilepsy in *Tsc1^{GFAP}*CKO mice without drug treatment. In previous studies, seizures were first observed between one to two months of age and appeared to become more frequent with age, but this was not formally analyzed (Uhlmann et al., 2002). In this study, EEG electrodes were implanted and 48 hr video-EEG monitoring sessions were started at different ages, ranging from 4 to 8 weeks. Performing video-EEG studies at ages younger than 4 weeks is difficult due to technical reasons. After the first 48 hr monitoring session, repeat 48 hr sessions were performed once a week on every subsequent week until the animal died or, rarely, a malfunction in the EEG electrodes occurred. A total of 15 *Tsc1^{GFAP}*CKO mice were utilized for these experiments and most were monitored on multiple successive weeks, but few spanned all time points analyzed in this study (4–9

weeks; 6–9 mice per time point). For each weekly time point, an average of interictal EEG background score, ratio of theta/delta power, spike frequency, and seizure frequency and duration was calculated as described above.

The second set of experiments tested the acute effects of standard antiepileptic drugs on EEG background activity and seizures in *Tsc1^{GFAP}CKO* mice. In this study, baseline video-EEG monitoring of mice typically started at 4–5 weeks of age and was repeated in 48 hr epochs, until the mice had at least 3 seizures during a 48 hr period. The mice were then randomly assigned to one of three treatment modalities (10–12 mice per group). Mice received daily intraperitoneal injections of either phenobarbital (50 mg/kg/d), phenytoin (40 mg/kg/d), or saline. The phenobarbital and phenytoin dosing paradigms were based on previous studies documenting therapeutic levels in rodents (Loscher and Honack, 1989; Rundfeldt and Loscher, 1993). Mice received daily injections for five days, the last 48 hours of which they received video-EEG monitoring to assess drug effects. Comparisons were made between pre-drug and drug monitoring sessions for interictal EEG background score, ratio of theta/delta power, spike frequency, and seizure frequency and duration, as described above. About half of the mice in the phenytoin and phenobarbital groups were euthanized after the last monitoring session (approximately six hours after the last drug injection) to obtain serum for drug level measurements (performed at the Saint Louis Children's Hospital Clinical Laboratory). The other half of the mice in these groups continued to receive daily injections of phenytoin or phenobarbital without subsequent video-EEG monitoring until they died, to assess possible effects of these drugs on long-term survival of the mice.

Statistical Analysis

One-way ANOVA with Tukey-Kramer post-tests for multiple comparisons was used to analyze differences in interictal EEG background score, ratio of theta/delta power, spike frequency, seizure frequency, and seizure duration, between different ages in the untreated mice. Paired t-tests were used to analyze differences in these same parameters comparing pre-drug and drug monitoring sessions for each drug group. Data are expressed as mean $\pm$ SEM. Statistical significance was set at a P value < 0.05 .

RESULTS

Natural History and Progression of Epilepsy in *Tsc1^{GFAP}CKO* mice

The interictal EEG background of *Tsc1^{GFAP}CKO* mice became progressively abnormal with age (Fig. 1). At 4 weeks of age, mice generally had a normal EEG background characterized by a typical “theta” rhythm (grade 1), with an average interictal grade of 1.2 ± 0.2 (Fig. 1A,B). Over the following several weeks, there was a gradual loss of the normal background activity and a progressive increase in the frequency of interictal epileptiform spikes (Fig. 1C). By 9 weeks of age, the interictal background activity often showed a burst-suppression pattern (grade 4) and average interictal grade had significantly increased to 2.6 ± 0.4 ($p < 0.01$ by ANOVA, $n = 6–9$ mice per time point). Consistent with the interictal grading system, quantitative power analysis by Fast Fourier Transform also demonstrated a change in the interictal EEG background activity with age. While higher frequencies did not vary significantly with age, theta (4–8 Hz) frequencies predominated in younger mice between 4–6 weeks of age and slower delta (1–4 Hz) frequencies were more common in older 8–9 week old mice (Fig. 1D). The ratio of theta/delta power was significantly decreased in the 8–9 week old mice (Fig. 1E; $p < 0.001$ by ANOVA, $n = 6–9$ mice per time point).

Seizures also became progressively more frequent with age (Fig. 2A,B). At 4 weeks of age, no seizures were recorded in most mice (67%) and the overall seizure frequency was only 1.0 ± 0.6 seizures/day. By 9 weeks of age, all mice recorded had seizures and the average

seizure frequency had significantly increased to 9.5 ± 3.5 seizures/day ($p < 0.05$ by ANOVA, $n = 6-9$ mice per time point). Although there appeared to be a non-significant “trend” toward longer seizures in 9 week old mice, average seizure duration did not change significantly with age (Fig. 2C). On video analysis, the clinical features of most seizures were typically characterized by an initial phase of behavioral arrest followed by progressive clonus of the forelimbs and occasional rearing, but no generalized convulsive activity. On EEG analysis, all seizures appeared to be generalized based on bilateral neocortical electrodes (Fig. 2A).

Only a few mice survived to 10 weeks or beyond, preventing meaningful statistical evaluation of EEG data or seizure frequency beyond 9 weeks. Consistent with previous reports (Uhlmann et al., 2002), all mice died by 3 months of age. Although the specific cause of death is not known and mice rarely have been documented to die during a seizure, mice appear to become progressively “encephalopathic” (correlating with the abnormal EEG background), with a dramatic decrease in normal activity and feeding behavior.

Treatment of Seizures with Standard Antiepileptic Drugs

Treatment with daily intraperitoneal injections of phenobarbital or phenytoin for 5 days significantly decreased the seizure frequency in *Tsc1^{GFAP}CKO* mice (Fig. 3A). In saline injected mice, the seizure frequency was not significantly different between pre-drug and drug monitoring periods (2.7 ± 0.3 seizures/day in pre-drug period versus 2.7 ± 0.8 in drug period; $n=12$ mice). In contrast, phenobarbital caused an almost 70% decrease in seizure frequency (2.8 ± 0.3 seizure/day in pre-drug period versus 0.8 ± 0.3 in drug period; $p < 0.01$ by paired t-test, $n=11$ mice), although only 36% became seizure-free in the drug period. Phenytoin caused an approximately 55% decrease in seizure frequency (3.1 ± 0.6 seizures/day in pre-drug period versus 1.4 ± 0.5 in drug period; $p < 0.05$ by paired t-test, $n=10$ mice), with 30% becoming seizure free. The average serum phenobarbital level was 38.1 ± 8.2 $\mu\text{g/ml}$ and phenytoin level was 16.6 ± 5.0 $\mu\text{g/ml}$ ($n=5$ for both drugs).

Despite the significant effects of both drugs on seizure frequency, phenobarbital and phenytoin did not have any significant effect on seizure duration (Fig. 3B) and frequency of interictal spikes (Fig. 3C). Phenobarbital and phenytoin treatment also did not significantly alter interictal background EEG score (Fig. 3D) or the frequency distribution of the interictal EEG by Fast Fourier Transform (ratio of theta/delta power = 1.03 ± 0.07 during phenobarbital treatment vs. 1.02 ± 0.07 in the pre-drug period; ratio of theta/delta = 0.98 ± 0.12 during phenytoin treatment vs. 1.09 ± 0.16 in the pre-drug period). Furthermore, mice that continued to receive daily phenobarbital ($n=6$) or phenytoin ($n=5$) injections all died within 20 days of the last monitoring session similar to saline-injected mice, indicating that acute treatment with these drugs did not affect long-term survival.

DISCUSSION

Given the severity and intractability of epilepsy in many TSC patients, novel therapies are clearly needed for epilepsy in TSC. The development and testing of new, more effective therapies would greatly benefit from an appropriate animal model, but most animal models of TSC have not been reported to have epilepsy. Although preliminary reports suggest that the neuronal-selective *Tsc1*-synapsin-Cre conditional knock-out mice may also have seizures at a very young age (Meikle et al. 2005), to our knowledge *Tsc1^{GFAP}CKO* mice presently may be the only animal model of TSC definitively documented to have epilepsy by EEG. In the present study, we have characterized the natural history and progression of epilepsy, as well as background interictal EEG abnormalities, in *Tsc1^{GFAP}CKO* mice in more detail and performed initial preclinical studies demonstrating the acute effects of two standard antiepileptic drugs on seizures in these mice. This study also validates the

Tsc1^{GFAP}CKO mouse as a preclinical model for assessing drug efficacy for epilepsy in TSC and should serve as a baseline and reference for future trials testing novel mechanistically-based drugs that may be more effective in preventing or reversing epileptogenesis in TSC.

Previous studies have documented the presence of seizures in *Tsc1*^{GFAP}CKO mice by video-EEG and suggested a delayed onset and progressive course of the epilepsy, but a detailed time course was not systematically studied (Uhlmann et al., 2002). In the present study, we have confirmed that epilepsy in *Tsc1*^{GFAP}CKO mice typically starts around one to two months of age and the seizures become progressively more frequent over the ensuing month, culminating in death by three months of age. The interictal EEG background showed a corresponding evolution, starting with a basically normal EEG at one month and progressing to a terminal “burst-suppression” pattern. A limitation of this study is the lack of chronic EEG recordings in mice younger than 4 weeks of age, as long-term video-EEG monitoring necessary to document spontaneous seizure frequency is extremely difficult in younger mice due to technical reasons (e.g. softness of skull reducing electrode stability; necessary presence of the maternal dam for pre-weanling mice). Thus, it is difficult to absolutely exclude the possibility that seizures occur at a much younger age than documented in the present study. However, there are several reasons to suggest that this possibility is unlikely, or if seizures do occur at a younger age, they are very rare. First, only rare seizures are observed in a minority of mice monitored by video-EEG at 4 weeks of age and there is a dramatic, progressive increase in the new incidence and frequency of seizures after 6 weeks of age (Fig. 2B). Second, assuming that interictal EEG background abnormalities correlate with seizure frequency in untreated mice (compare Fig. 1B and 2B), the essentially normal interictal EEG background of 4 week old mice suggests that seizures are very rare prior to this age. Finally, gross behavioral observations have never documented signs of clinical seizure activity in pre-weanling *Tsc1*^{GFAP}CKO mice (Zeng and Wong, unpublished observations). Thus, we believe that the data in this study presents an accurate picture of the developmental trajectory of epilepsy in *Tsc1*^{GFAP}CKO mice.

This documentation of the natural history of seizures in *Tsc1*^{GFAP}CKO mice is important for determining potential molecular, cellular, and anatomical mechanisms of epileptogenesis, as relevant causative mechanisms should temporally coincide with or precede the development of seizures. Previous studies have identified molecular abnormalities in astrocytic glutamate transporters and potassium channels as candidate mechanisms for promoting epileptogenesis in *Tsc1*^{GFAP}CKO mice (Wong et al., 2003; Jansen et al., 2005). These astrocyte defects have been documented in brain slices from 2–3 week old *Tsc1*^{GFAP}CKO mice, as well as in cultured astrocytes derived from neonatal mice, indicating that these molecular abnormalities precede the development of epileptiform abnormalities as seen in the present study and thus could represent primary causative mechanisms involved in the early stages of epileptogenesis. In contrast, the cellular and histological abnormalities of increased brain size, astrocyte proliferation, and neuronal disorganization usually start to become evident after 3–4 weeks of age (Uhlmann et al., 2002), which likely reflects later stages of epileptogenesis. In addition to allowing correlations with potential mechanisms of epileptogenesis, the present description of the natural history of epilepsy in *Tsc1*^{GFAP}CKO mice should serve as an important comparative baseline for future studies testing the efficacy of drugs at an early age that can potentially prevent or delay epileptogenesis.

The initial drug trials in this study tested the effectiveness of two commonly-used antiepileptic drugs on seizures and interictal EEG abnormalities in *Tsc1*^{GFAP}CKO mice. Acute, short-term treatment with phenytoin or phenobarbital at a relatively late stage of epileptogenesis (after seizure onset) significantly decreased seizure frequency in *Tsc1*^{GFAP}CKO mice, supporting the known efficacy of these drugs as anticonvulsants. However, these drugs did not appear to improve the underlying interictal EEG abnormalities

or ultimately prevent death in these mice. It is possible that future studies involving earlier treatment with these drugs starting in the neonatal or juvenile period before the onset of epileptogenesis might have better results on overall prognosis. However, based on negative results of human trials of antiepileptic drugs for preventing chronic epilepsy following head trauma (Temkin et al., 1990), it seems more likely that most currently available “antiepileptic” drugs simply suppress seizures in the short-term but do not alter the underlying process of epileptogenesis.

Although the clinical applications of the drug trials from the present study may be somewhat limited in scope, this study serves as an important “proof-of-principle” of the utility of *Tsc1^{GFAP}*CKO mice as a preclinical animal model for drug testing for epilepsy in TSC. An essential element of validating small-animal models of human disease is demonstrating that the phenotypes observed in genetically-engineered mice respond similarly to treatments effective in people. In this regard, we found that seizures in this murine epilepsy model of TSC are attenuated by two antiepileptic drugs commonly used in clinical practice. Moreover, to our knowledge, this is the first reported preclinical treatment study of epilepsy in an animal model of TSC. The results from this study lay the foundations for future preclinical studies of drugs that target the molecular basis of *TSC* loss in the brain.

Future drug development in epilepsy is focused on finding drugs with true “anti-epileptogenic” properties. In TSC, strong candidates include drugs that modulate upstream signaling pathways that are abnormally active due to mutations in the *TSC* genes, such as the mammalian target of rapamycin (mTOR; Gao et al., 2002; Inoki et al., 2002; Tee et al., 2002; Uhlmann et al., 2004). Furthermore, based on our previous work implicating impaired astrocyte glutamate transporters and potassium channels in epileptogenesis in *Tsc1^{GFAP}*CKO mice (Wong et al., 2003; Jansen et al., 2005), drugs that regulate expression of these astrocyte transporters and channels (Rothstein et al., 2005) may prove to be rational therapies for epilepsy in TSC. Thus, *Tsc1^{GFAP}*CKO mice represent an ideal model for future studies testing the effect of mTOR inhibitors and other potential “anti-epileptogenic” drugs on epilepsy in TSC.

Acknowledgments

This work was supported by the National Institutes of Health (K02NS045583 and R01NS056872, MW) and the Tuberous Sclerosis Alliance (MW).

REFERENCES

- Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *New England Journal of Medicine*. 2006; 355:1345–1346. [PubMed: 17005952]
- Ess KC, Uhlmann EJ, Li W, Li H, Declue JE, Crino PB, Gutmann DH. Expression profiling in tuberous sclerosis complex (TSC) knockout mouse astrocytes to characterize human TSC brain pathology. *Glia* 2004. 2004; 46:28–40.
- Gao X, Zhang Y, Arrazola P, Hino O, Kobayashi T, Yeung RS, Ru B, Pan D. TSC tumor suppressor proteins antagonize amino-acid-TOR signaling. *Nature Cell Biology*. 2002; 4:699–704.
- Griffey MA, Wozniak D, Wong M, Bible E, Johnson K, Rothman SM, Wentz A, Cooper JD, Sands M. CNS-directed AAV2-mediated gene therapy ameliorates functional deficits in a murine model of infantile neuronal ceroid lipofuscinosis. *Molecular Therapy*. 2006; 13:538–547. [PubMed: 16364693]
- Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signaling. *Nature Cell Biology*. 2002; 4:648–657.
- Jansen LA, Uhlmann EJ, Gutmann DH, Wong M. Epileptogenesis and reduced inward rectifier potassium current in *Tuberous Sclerosis Complex-1* deficient astrocytes. *Epilepsia*. 2005; 46:1871–1880. [PubMed: 16393152]

- Kielar C, Maddox L, Bible E, Pontikis CC, Macauley SL, Griffey MA, Wong M, Sands MS, Cooper JD. Successive neuron loss in the thalamus and cortex in a mouse model of infantile neuronal ceroid lipofuscinosis. *Neurobiology of Disease*. 2007; 25:150–162. [PubMed: 17046272]
- Kwiatkowski DJ. Tuberous sclerosis: from tubers to mTOR. *Annals of Human Genetics*. 2003; 67:87–96. [PubMed: 12556239]
- Loscher W, Honack D. Comparison of the anticonvulsant efficacy of primidone and phenobarbital during chronic treatment of amygdala-kindled rats. *European Journal of Pharmacology*. 1989; 162:309–322. [PubMed: 2721568]
- Meikle LM, Talos DM, Onda H, Walker V, Zhang H, Jensen FE, Kwiatkowski DJ. Characterization of a murine neuronal specific knockout of Tsc1. *Epilepsia*. 2005; 46(Suppl.5):273.
- Mizuguchi M, Takashima S, Yamanouchi H, Nakazato Y, Mitani H, Hino O. Novel cerebral lesions in the Eker rat model of tuberous sclerosis: cortical tuber and anaplastic ganglioglioma. *Journal of Neuropathology and Experimental Neurology*. 2000; 59:188–196. [PubMed: 10744057]
- Mizuguchi M, Takashima S. Neuropathology of tuberous sclerosis. *Brain and Development*. 2001; 23:508–515. [PubMed: 11701246]
- Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DL, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature*. 2005; 43:73–77. [PubMed: 15635412]
- Rundfelt C, Loscher W. Anticonvulsant efficacy and adverse effects of phenytoin during chronic treatment in amygdala-kindled rats. *Journal of Pharmacology and Experimental Therapeutics*. 1993; 266:216–223. [PubMed: 8331559]
- Sparagana SP, Roach ES. Tuberous sclerosis complex. *Current Opinion in Neurology*. 2000; 13:115–119. [PubMed: 10987566]
- Takahashi DK, Dinday MT, Barbaro NM, Baraban SC. Abnormal cortical cells and astrocytomas in the Eker rat model of tuberous sclerosis complex. *Epilepsia*. 2004; 45:1525–1530. [PubMed: 15571510]
- Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis J. Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *Proceedings of the National Academy of Science USA*. 2002; 99:13571–13576.
- Temkin NR, Dikmen SS, Wilensky AJ, Keihm J, Chabal S, Winn HR. A randomized, double-blind study of phenytoin for the prevention of post-traumatic seizures. *New England Journal of Medicine*. 1990; 323:497–502. [PubMed: 2115976]
- Tschuluun N, Wenzel HJ, Schwartzkroin PA. Irradiation exacerbates cortical cytopathology in the Eker rat model of tuberous sclerosis complex, but does not induce hyperexcitability. *Epilepsy Research*. 2007 in press.
- Uhlmann EJ, Wong M, Baldwin RL, Bajenaru ML, Onda H, Kwiatkowski DJ, Yamada KA, Gutmann DH. Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. *Annals of Neurology*. 2002; 52:285–296. [PubMed: 12205640]
- Uhlmann EJ, Li W, Scheidenhelm DK, Gau CL, Tamanoi F, Gutmann DH. Loss of tuberous sclerosis complex 1 (Tsc1) expression results in increased Rheb/S6K pathway signaling important for astrocyte cell size regulation. *Glia*. 2004; 47:180–188. [PubMed: 15185396]
- Von der Brélie C, Waltereit R, Zhang L, Beck H, Kirschstein T. Impaired synaptic plasticity in a rat model of tuberous sclerosis. *European Journal of Neuroscience*. 2006; 23:686–692. [PubMed: 16487150]
- Waltereit R, Welzl H, Dichgans J, Lipp HP, Schmidt WJ, Weller M. Enhanced episodic-like memory and kindling epilepsy in a rat model of tuberous sclerosis. *Journal of Neurochemistry*. 2006; 96:407–413. [PubMed: 16300636]
- Wenzel HJ, Patel LS, Robbins CA, Emmi A, Yeung RS, Schwartzkroin PA. Morphology of cerebral lesions in the Eker rat model of tuberous sclerosis. *Acta Neuropathologica*. 2004; 108:97–108. [PubMed: 15185103]

Wong M, Ess KE, Uhlmann EJ, Jansen LA, Li W, Crino PB, Mennerick S, Yamada KA, Gutmann DH. Impaired astrocyte glutamate transport in a mouse epilepsy model of tuberous sclerosis complex. *Annals of Neurology*. 2003; 54:251–256. [PubMed: 12891680]

Figure 1.

The interictal EEG activity of *Tsc1*^{GFAP}CKO mice becomes progressively abnormal with age. (A) Representative examples of the 4 interictal EEG background grades, as described in the Methods, are shown: Grade 1 – normal background activity ($\pm$ 6-8 Hz sinusoidal theta rhythm), no epileptiform spikes; 2 – mostly normal background activity, few epileptiform spikes; 3 – mostly abnormal background activity, many spikes; 4 – burst-suppression pattern. (B) Average interictal EEG background grade of *Tsc1*^{GFAP}CKO mice shows progressive worsening with age ($p < 0.01$ by ANOVA; *post-test comparisons show weeks 8 and 9 to be significantly greater than week 4; $n = 6-9$ mice per time point). (C) Average interictal spike frequency of *Tsc1*^{GFAP}CKO mice also increased with age ($p < 0.001$ by ANOVA; *post test comparisons show weeks 7-9 to be significantly greater than weeks 4

and 6; n = 6-9 mice per time point). (D) Fast Fourier Transform of interictal EEG background of *Tsc1*^{GFAP}CKO mice reveals different frequency distributions dependent on age. Average power spectra for 4 week and 9 week old mice are shown. Theta (4-8 Hz) frequencies predominated in 4 week old mice and slower delta (1-4 Hz) frequencies were more common in older 9 week old mice (For clarity, data for 5-8 week old mice are not shown). (E) The ratio of theta/delta power decreased with age (p<0.001 by ANOVA; *post test comparisons show weeks 8 and 9 to be significantly less than weeks 4-6; n = 6-9 mice per time point).

Figure 2.

The progression of epilepsy in *Tsc1*^{GFAP}CKO mice. (A) A representative example of ictal EEG activity recorded during a typical seizure. Seizures usually started with bilateral fast tonic activity that evolved into higher amplitude spikes. The end of the seizure typically evolved into bursting spike discharges, followed by postictal suppression. (B) Average seizure frequency of *Tsc1*^{GFAP}CKO mice increased with age ($p < 0.05$ by ANOVA; *post test comparisons show week 9 to be significantly greater than weeks 4-6; $n = 6-9$ mice per time point). (C) Average seizure duration did not change with age. Data for weeks 4-6 were combined due to the small number of mice with seizures at these ages. Seizure duration

appeared to be higher at 9 weeks, but this difference was not statistically significant ($p = 0.08$ by ANOVA; $n = 6-9$ mice per time point).

Figure 3.

Acute treatment with phenobarbital or phenytoin reduces seizure frequency, but does not affect seizure duration or interictal EEG background. (A) There was no significant difference in seizure frequency between pre-drug and drug periods in saline-injected *Tsc1^{GFAP}CKO* mice, but both phenobarbital and phenytoin significantly reduced seizure frequency in *Tsc1^{GFAP}CKO* mice (* $p < 0.01$ for phenobarbital, $p < 0.05$ for phenytoin by paired t-tests; $n = 10-11$ mice per group). (B-D) Phenobarbital and phenytoin had no significant effect on seizure duration, interictal spike frequency or interictal EEG background grade ($p > 0.05$ for all comparisons; $n = 10-11$ mice per group). Pre – pre-drug monitoring period, Sal- saline, PB – phenobarbital, PHT – phenytoin.