Published in final edited form as:

Exp Neurol. 2016 May ; 279: 116–126. doi:10.1016/j.expneurol.2016.02.014.

Repeated low-dose kainate administration in C57BL/6J mice produces temporal lobe epilepsy pathology but infrequent spontaneous seizures

Anthony D. Umpierre^a, Isaiah V. Bennett^b, Lismore D. Nebeker^b, Thomas G. Newell^{c,d}, Bruce B. Tian^b, Kyle E. Thomson^{c,d}, H. Steve White^{b,c}, John A. White^e, and Karen S. Wilcox^{a,b,c,*}

^a Interdepartmental Program in Neuroscience, University of Utah, 401 MREB, 20N. 1900E., Salt Lake City, UT 84132

^b Dept. of Pharmacology & Toxicology, University of Utah, College of Pharmacy, 30N. 1900E., Salt Lake City, UT 84112

^c Anticonvulsant Drug Development Program, University of Utah, 417 Wakara Way, Suite 3211, Salt Lake City, UT 84108

^d Dept. of Bioengineering, University of Utah, 36S. Wasatch Drive, Rm. 3100, Salt Lake City, UT 84112

^e Dept. of Biomedical Engineering, Boston University, 44 Cummington Mall, Rm. 403, Boston, MA 02215

Abstract

More efficient or translationally relevant approaches are needed to model acquired temporal lobe epilepsy (TLE) in genetically tractable mice. The high costs associated with breeding and maintaining transgenic, knock-in, or knock-out lines places a high value on the efficiency of induction and animal survivability. Herein, we describe our approaches to model acquired epilepsy in C57BL/6J mice using repeated, low-dose kainate (KA) administration paradigms. Four paradigms (i.p.) were tested for their ability to induce status epilepticus (SE), temporal lobe pathology, and the development of epilepsy. All four paradigms reliably induce behavioral and/or

*Corresponding Author: Karen S. Wilcox, Ph.D., Department of Pharmacology & Toxicology, University of Utah, 201 Skaggs Hall, Salt Lake City, UT 84112, Phone: (801)-581-4081, Karen.Wilcox@hsc.utah.edu.

Anthony D. Umpierre: Anthony.Umpierre@utah.edu

Isaiah V. Bennett: isaiahvbennett98@gmail.com

Lismore D. Nebeker: U0879974@utah.edu

Thomas G. Newell: Thomas.Newell@utah.edu

Bruce B. Tian: U0667419@utah.edu

Kyle E. Thomson: K.Thomson@utah.edu

H. Steve White: Steve.White@hsc.utah.edu

John A. White: jwhite@bu.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Statement:

--The authors declare no conflict(s) of interest--

electrographic SE without mortality over a 7d period. Two of the four paradigms investigated produce features indicative of TLE pathology, including hippocampal cell death, widespread astrogliosis, and astrocyte expression of mGluR5, a feature commonly reported in TLE models. Three of the investigated paradigms were able to produce aberrant electrographic features, such as interictal spiking in cortex. However, only one paradigm, previously published by others, produces spontaneous recurrent seizures over an eight week period. Presentation of spontaneous seizures is rare (N=2/14), with epilepsy preferentially developing in animals having a high number of seizures during SE. Overall, repeated, low-dose KA administration improves the efficiency and pathological relevance of a systemic KA insult, but does not produce a robust epilepsy phenotype under the experimental paradigms described herein.

Keywords

Temporal lobe epilepsy; Seizure; C57 (C57BL/6J); Kainate; Valproic acid; Astrogliosis; mGluR5; Cell death; Neurodegeneration

Introduction

Status epilepticus (SE) is a life-threatening, prolonged seizure event of multiple etiologies (Trinka et al., 2015). Although the annual incidence rate of SE is low (40 in 100,000; (Trinka et al., 2012)), SE is considered a risk factor for acquired epilepsy (Hesdorffer et al., 1998). The acquisition of TLE following a brain insult is likely to occur through a number of complex mechanisms (Gorter et al., 2015; Henshall and Engel, 2015; Wilcox et al., 2015). The use of transgenic, knock-in, and knock-out approaches, often developed or available on a C57BL/6 (C57) background, can greatly aid in disentangling the complexity of epileptogenesis; however, modeling epileptogenesis in C57 mice has not been straightforward.

Systemic administration of chemoconvulsants (pilocarpine or kainate (KA)) has served as an efficient means to model epilepsy development in rat; however, chemconvulsants display a particularly poor response profile in C57BL/6 substrains. In the more extensively used pilocarpine model of SE, certain substrains of C57 mice are resistant to pilocarpine, while other substrains exhibit an extremely narrow dose-dependent range of SE susceptibility. As a consequence, high rates of mortality or low rates of SE induction have been reported in the pilocarpine mouse model (Borges et al., 2003; Muller et al., 2009; Oliveira et al., 2015). The identification of pilocarpine-responsive candidate genes or substrains (Bankstahl et al., 2012; Winawer et al., 2011; Winawer et al., 2014) in combination with repeated, low-dose injection paradigms (Groticke et al., 2007) has improved the efficacy SE induction in the pilocarpine mouse model.

Notable concerns and questions still remain for the KA mouse model. Single, bolus doses of KA result in variable rates of mortality and SE induction (Hu et al., 1998; McCord et al., 2008). Additionally, bolus doses of KA do not typically produce hippocampal cell death in C57 mice, despite the induction of SE (Chang et al., 2012; McCord et al., 2008; Schauwecker, 2000, 2003; Schauwecker and Steward, 1997), but see (Chang et al., 2012; Lee et al., 2014). Seizure-induced cell death in hippocampus may contribute to the

hippocampal sclerosis commonly observed in patients with temporal lobe epilepsy (TLE) (Thom, 2009), which is modeled well in rat following either pilocarpine- or KA-induced SE (Polli et al., 2014). However, no study to date has detailed the chronic impact of systemic KA-induced SE in C57 mice, including rates of epilepsy development, although isolated observations of spontaneous seizures have been reported (Schauwecker and Steward, 1997).

In an attempt to investigate the potential concerns and questions surrounding systemic KA insults in C57 mice, we evaluated multiple low-dose KA administration paradigms as a means to induce SE, produce relevant pathology, and limit acute mortality. We observe that systemic, low-dose KA administration (i.p.) reliably produces SE without acute mortality (7d), similar to reports by Tse et al. (2014). Additionally, low-dose KA insults result in features indicative of TLE pathology, including hippocampal neurodegeneration and astrogliosis. Systemic, low-dose KA paradigms were further tested for their ability to induce epilepsy. Only one out of the three paradigms investigated produced spontaneous, recurrent seizures. Animals displaying spontaneous, recurrent seizures experienced an unusually high seizure burden during SE.

Materials and methods

Animals

Male C57BL/6J mice were acquired from Jackson Laboratories (Bar Harbor, ME) at 5-6 weeks of age to maintain consistency with previous studies of acquired epilepsy (Stewart et al., 2009; Stewart et al., 2010; Umpierre et al., 2014). Animals were maintained in temperature- and humidity-controlled rooms on a 12-h light-dark cycle (lights on at 6am), and provided free access to food and water. Mice were group housed for acute studies, or singly housed for chronic monitoring after the placement of a cortical electrode. All procedures performed in this study adhered to the standards of the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Utah Institutional Animal Care and Use Committee (IACUC).

Repeated low-dose KA injection and behavioral seizure monitoring

After a 3-5 d acclimation period, a subset of mice were systemically injected (i.p.) with KA following one of four dosing paradigms. Kainic acid (Tocris Bioscience, Bristol, UK) was dissolved overnight in sterile isotonic saline prior to an experiment and kept for use no longer than 7d. To reduce variability, all solutions were made from the same vial of kainic acid. Valproic acid (VPA; Sigma-Aldrich, St. Louis, MO) was used to reduce seizure activity in a subset of paradigms, since pharmacoresistance to benzodiazepines can occur during SE (Mayer et al., 2002; Naylor et al., 2005).

In the “protracted KA/VPA” injection paradigm, animals (N=10; weight: 18-22g) received an initial 5 mg/kg injection of KA followed by repeated 2.5 mg/kg injections, all separated by 20 minutes, until the first Racine stage 4/5 seizure was observed (Racine, 1972), defined as forelimb clonus with rearing (stage 4), possibly escalating into loss of postural control (stage 5). Any animal displaying two additional Racine stage 4/5 seizures within 90 minutes of the first seizure was considered to have undergone behavioral SE (Shibley and Smith,

2002)—inclusion criteria for histological and immunofluorescent analyses. Ninety minutes after the first Racine stage 4/5 seizure was observed, seizure activity was reduced by VPA (300 mg/kg, i.p.). For behavioral seizure monitoring, seizures were not scored by a reviewer blinded to paradigm.

In the “expedited KA/VPA (ex-KA/VPA)” or “expedited KA/NaCl (ex-KA/NaCl)” injection paradigms, animals (weight: 19-22g) received an initial 10 mg/kg injection of KA, a 5 mg/kg injection, and then repeated 2.5 mg/kg injections until the first Racine stage 4/5 seizure was observed. All injections were separated by 20 min. The criteria for behavioral SE remained identical to the protracted KA/VPA paradigm. However, after 90 minutes of SE, animals were either injected with VPA (300 mg/kg, i.p.; ex-KA/VPA paradigm, N=6) or sterile saline (200µl, i.p.; ex-KA/NaCl paradigm, N=10).

Finally, we tested a previously published low-dose KA paradigm with modifications. In this “5 mg/kg” injection paradigm, animals (N=3; weight: 20-24g) received 5 mg/kg injections of KA every 20 minutes until an initial Racine stage 5 seizure was observed, modified from (Tse et al., 2014). Animals displaying an initial Racine stage 3 seizure received a 2.5 mg/kg injection if a Racine stage 4/5 seizure was not observed before the time of next injection. To enhance and prolong SE, animals that did not display a behavioral seizure 45-60 minutes after an initial seizure were given a final 2.5 mg/kg booster dose of KA. No anti-seizure drug was administered in this paradigm.

Age-matched control animals (NaCl/VPA, N=5) received an equivalent number of 100µl injections (i.p.) of sterile isotonic saline, which approximated the fluid volume of KA injections. Control animals were injected with VPA (300 mg/kg, i.p.) 2h after an initial saline injection.

For all paradigms, lactated Ringer's solution (500ul, s.c.) was administered to control and KA-treated mice two hours after the final injection and on any day in which body weight had dropped 0.5g compared to the previous day.

EEG implant for acute and chronic monitoring

A subset of mice (weight range: 20-28g) was anesthetized with ketamine/xylazine anesthesia (80 mg/kg ketamine, 12 mg/kg xylazine, i.p.) and a cortical electrode (Plastics One, Roanoke, VA) was placed above the left parietal cortex, or a bipolar depth electrode was placed into the CA1 cell body layer of ventral hippocampus (anteroposterior (AP): -2.7; mediolateral (ML): +2.0; dorsoventral (DV): -1.5). Electrodes were secured by super glue (Loctite 454, Westlake, OH) against three anchor screws. Animals were allowed 7-14d to recover. During recording, electrodes were connected via flexible tether to a rotating commutator (Plastics One) connected to an EEG100C amplifier (BioPac Systems, Goleta, CA), which filtered signal below 1Hz and above 100Hz. Custom programming (Thomson and White, 2014) allowed for continuous EEG data to be synchronized in real time with video monitoring of animal behavior and written to disk for later review.

To monitor electrographic SE, one hour of baseline EEG was recorded before cohorts were injected with KA according to the ex-KA/VPA, ex-KA/NaCl, or 5 mg/kg KA paradigm (Tse

et al., 2014). Changes in electrographic power (1-100Hz, 90s window, 45s step) during KA injections were plotted across a 24h period that included 1h of baseline activity used to normalize EEG power spectra. Post-processing was performed in MATLAB (MathWorks, Natick, MA), using the Chronux Toolbox (<http://chronux.org/>). Additionally, a trained reviewer, blinded to paradigm, counted and scored corresponding behavioral seizures during SE using standard Racine stage 1-5 scoring criteria. The number of convulsive motor seizures (Racine stage 3-5) and the seizure burden (aggregate of all Racine behavioral seizure scores) was calculated over a 24h period beginning at the time of first injection.

Video EEG monitoring persisted for 7-8 weeks and was reviewed for spontaneous recurrent seizures by a reviewer blinded to kainate paradigm. A second, reviewer (not blinded) confirmed spontaneous seizures. Age-matched animals injected with saline and VPA (NaCl/VPA) served as controls across an identical period. An experienced reviewer manually investigated EEG traces for the presence of interictal spikes, defined as transient electrographic events 3x the baseline signal amplitude. A clustering of >10 spikes within a 5 minute period was common to observe and served as the criteria for designating an animal as spiking or non-spiking within a 12-h period.

Tissue processing for staining

A subset of animals that underwent behavioral SE (**Table 1**) were sacrificed 7d after SE. Briefly, animals were deeply anesthetized with 60 mg/kg pentobarbital (i.p.) and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. Whole brains were removed and allowed to post-fix in 4% PFA for 6-8 h at 4°C before being transferred to a 15/30% sucrose solution for overnight cryoprotection. Coronal sections containing the medial and ventral hippocampus were sliced on a freezing stage microtome and stored at 4°C in 0.1M PBS for staining.

Histology, Immunofluorescence, and Imaging

FluoroJade-B staining—FluoroJade-B (FJB) staining was performed using two medial hippocampal slices per animal and batch processed similarly to published methods (McCord et al., 2008), except sections were blocked for 27 min. in 0.06% KMnO₄ and stained for dying neurons for 15 min using a 0.0004% FJB solution. Images were acquired using a Zeiss Axio Imager A1 microscope at 20x magnification. The DAPI filter was used to identify regions of interest before images were acquired using the TRITC filter (FJB). After acquisition, images of hippocampal subregions were masked such that they only contained stratum pyramidale (CA3 or CA1) or the hilus. No masking was performed in the thalamic or amygdala regions. FJB-positive area was quantified in Image-J through the following procedures: files were converted to 8-bit black and white images, pixel intensity was inverted, a uniform threshold was established, and a pixel to micron scale factor was set in order to quantify the FJB-positive pixel area per image using the ‘analyze particles’ tool.

mGluR5 and GFAP staining—For immunofluorescent (IF) staining, sections were plated in 0.1M PBS and allowed to dry before permeabilizing for 1h in 0.3% Triton X-100 in PBS. For colocalization studies, sections (N=2/animal) were washed once in PBS and exposed to mGluR5 primary antibody (Rabbit polyclonal mGluR5, AB5675, Millipore;

1:500 in Cyto-Q) or Cyto-Q alone (primary omission) overnight at 4°C. For astrogliosis studies, sections (N=2/animal) were exposed to Cyto-Q overnight. Excess primary antibody was removed by PBS washes and secondary antibodies were applied for 1.5h at room temperature. For colocalization studies, an AlexaFluor 488 antibody was used to detect the mGluR5 primary antibody (goat anti-rabbit, 1:1000 in PBS; A11034, Life Technologies, Grand Island, NY) and a pre-conjugated GFAP antibody was used to detect astrocytes (GFAP-Cy3, 1:1000 in PBS, C9205, Sigma). For astrogliosis studies, tissue was processed with pre-conjugated GFAP-Cy3 antibody alone (Sigma). Secondary antibodies were removed by repeated PBS washes before applying DAPI for 10 minutes as a counterstain. A final PBS wash was performed before using Pro-Long Gold to mount slides.

Sections were imaged using a Nikon A1 confocal microscope at 20x resolution. For colocalization studies, laser power for the mGluR5 channel was set at a level at which virtually no signal could be detected in primary omission slides. Using primary omission slides, a colocalization threshold was set as the average colocalized area between the GFAP and AlexaFluor 488 in the absence of mGluR5 primary antibody. This value represents the level of aberrant colocalization due to non-specific secondary staining. For astrogliosis and colocalization studies, laser power for the GFAP channel was set at a level which best produced a linear range of signal between control and KA-lesioned tissue. Regions of interest were pre-selected using the DAPI channel before acquiring z-stacks (1 μ m step size). Z-stacks were converted to 8-bit tiff images for each channel and processed using Image-J. We used a previously established method for quantifying receptor colocalization with astrocytes (Vargas et al., 2013). GFAP staining was processed and quantified identically to FluoroJade-B staining in Image-J using the 'analyze particles' tool, except the regions surrounding the CA3 and CA1 cell body layers were not masked.

Statistics—Given our goal of determining pathological changes between KA and control animals, non-seized animals were not included in the ultimate statistical design. Due to the prolonged time course of inducing SE under the protracted KA/VPA paradigm, the paradigm was not adopted for future studies and was not considered in the statistical design. For analyses of astrogliosis, cell death (FluoroJade-B), and mGluR5 colocalization, a one-way ANOVA design was utilized with a Dunnett's post-hoc comparison to controls. Given the variability inherent in pathology, results are displayed as a box-and-whisker distribution, containing the minimum, maximum, and median of each group, plotted on a logarithmic scale ($\log_{10}$). A p-value below 0.05 was considered significant in post-hoc testing. All statistical analyses were performed using Prism 5 software (GraphPad, La Jolla, CA).

Results

Establishing an efficient injection paradigm for KA-induced SE

Previous studies inducing SE in C57 mice utilized a single bolus dose of KA administered systemically (Hu et al., 1998; Schauwecker and Steward, 1997). In an initial cohort (N=10), a single dose of 25 mg/kg KA (i.p.), just below a commonly used dose of 30 mg/kg (Schauwecker and Steward, 1997) was administered to C57BL/6J mice. Unexpectedly, a 25 mg/kg dose of KA proved fatal (N=10/10) after animals entered into continuous SE.

In response, multiple repeated, low-dose KA administration paradigms were attempted. All paradigms are described in **Table 1** with a report of the cohort N, SE induction rate, time to first seizure, aggregate dose of KA administered, and mortality rate. The first paradigm attempted, termed the “protracted KA/VPA” paradigm was modeled off of the dosing schedule used in low-dose KA rats (Hellier et al., 1998). Given the 120-140 min. latency to first seizure under the protracted KA/VPA paradigm, we also tested an expedited variant, termed the “ex-KA/VPA” paradigm, in which initial doses of KA were doubled to reach a first convulsive seizure in less time. An iteration of this paradigm without the use of VPA was also attempted, termed the “ex-KA/NaCl” paradigm. Notably the absence of VPA did not increase mortality over a 7d observational period. In general, all three paradigms tested for behavioral SE induction reliably induced SE (rate: 60-90%) without any acute mortality (N=26/26 animals surviving 7d).

Widespread cell death and astrogliosis results from low-dose KA administration

One week following low-dose KA administration, animals enrolled in the ex-KA/VPA and ex-KA/NaCl paradigms were sacrificed by transcardial perfusion and brain sections were assayed for pathological features. We first investigated whether repeated low-dose KA administration results in acute cell death following SE induction. Historically, a single bolus dose of KA, administered systemically to C57 animals, does not lead to cell death in hippocampus or other epilepsy-related brain regions (McCord et al., 2008; Schauwecker, 2000, 2003; Schauwecker and Steward, 1997). However, multiple brain regions in ex-KA/VPA and ex-KA/NaCl tissue displayed FJB-positive staining (**Fig. 1**). For ex-KA/VPA animals (N=5), FJB-positive staining was limited to hippocampal subregions, including the CA3 and CA1 cell body layers and the hilus (quantified in **Fig. 1I** against control (NaCl/VPA) levels). Without the administration of VPA, low-dose KA administration (ex-KA/NaCl) resulted in widespread degeneration throughout the coronal slice (**Fig. 1B**, representative image), with FJB-positive staining elevated above control levels in the CA3 and CA1 cell body layers of hippocampus, the amygdala, dorsal thalamus, and ventral thalamus (**Fig. 1 C-G**), but not in the hilus (**Fig. 1H**). The ex-KA/VPA and ex-KA/NaCl cohorts examined for pathological features were injected, on the same day, with the same KA solution, arguing that a difference in KA concentration does not underlie the observed differences in pathology. FJB-positive cells were not observed in sections from control animals (NaCl/VPA; N=5; **Fig. 1A**), or in KA-treated animals that did not display behavioral SE (data not shown; N=5).

In addition to cell death, astrogliosis is a hallmark feature of TLE. We quantified reactive astrogliosis as the GFAP-positive area observed in control, ex-KA/VPA, and ex-KA/NaCl animals (**Fig. 2**). The GFAP antigen was detected through IF techniques and batch processed to reduce experimental variability. Four regions of interest were selected, including the hilus, a region of cortex superior to the hippocampus, and the *stratum oriens* and *stratum radiatum* surrounding either the CA3 or CA1 cell body layer (**Fig. 2C**). In control tissue, no common features of reactive astrogliosis were observed, such as hypertrophy or loss of individual astrocyte domains (N=5; **Fig. 2A,C**). A lack of apparent astrogliosis in NaCl/VPA control tissue suggests that isolated VPA administration (300 mg/kg, i.p.) was not sufficient to induce astrogliosis. For ex-KA/VPA animals (N=5), astrocytes within the hippocampus

displayed moderate hypertrophy relative to controls (**Fig. 2C**); however, quantification of the GFAP-positive area in ex-KA/VPA tissue was not distinguishable from control levels in cortex, hilus, or surrounding the CA3 and CA1 pyramidal layers (**Fig. 2D-G**). By contrast, astrogliosis was apparent in ex-KA/NaCl animals (**Fig. 2B, C**). GFAP-positive area was increased above control levels within the hilus (**Fig. 2D**) and around the CA3 (**Fig. 2E**) and CA1 (**Fig. 2F**) cell body layers. GFAP-positive area was not significantly elevated in cortex (**Fig. 2G**).

mGluR5 expression following low-dose KA administration

In addition to hypertrophy, reactive astrocytes are noted to display altered expression of numerous surface receptors during astrogliosis. In particular, mGluR5 may serve as a unique marker of astrocyte pathology. Previous studies have suggested mGluR5 is not expressed by astrocytes after early development in the basal state (Morel et al., 2014; Sun et al., 2013). However, in epilepsy-related pathology, numerous colocalization studies have suggested hippocampal astrocytes increase mGluR5 expression following excitotoxic KA lesions (Aronica et al., 2000; Ferraguti et al., 2001; Ulas et al., 2000) and angular bundle stimulation (Aronica et al., 2000). In addition to its expression, the function of astrocyte mGluR5 signaling is implicated in the promotion of cell death following systemic pilocarpine administration (Ding et al., 2007). We determined whether astrocyte mGluR5 expression is increased in C57BL/6/J mice following low-dose KA administration using IF staining and colocalization analysis (**Fig. 3**). In following previous studies of astrocyte mGluR5 expression, we limited our analyses to hippocampal astrocytes surrounding the CA3 and CA1 pyramidal layers (i.e. astrocytes of the *stratum radiatum* and *stratum oriens*). Most control tissue (N=4/5) exhibited mGluR5:GFAP colocalization values within a range that was not distinguishable from an experimentally determined level of colocalization attributable to non-specific secondary antibody signal (primary omission slides; **Fig. 3A**, representative image). By contrast, all colocalization values from ex-KA/VPA and ex-KA/NaCl treated tissue were above the threshold for non-specific staining. Comparisons between control (NaCl/VPA) and ex-KA/VPA groups indicate mGluR5:GFAP colocalization is elevated among astrocytes surrounding the CA1 pyramidal layer, but not astrocytes surrounding the CA3 pyramidal layer (**Fig. 3C**). For ex-KA/NaCl animals, mGluR5:GFAP colocalization is significantly elevated among astrocytes surrounding both the CA3 and CA1 subfields (**Fig. 3C**; representative image, **Fig. 3B**).

Electrographic seizure activity and behavioral seizure severity during expedited KA administration

We implanted a cortical EEG above parietal cortex to record electrographic SE and long-term EEG activity in a second cohort of ex-KA/VPA and ex-KA/NaCl animals for a seven week study (**Table 2**). Previously, it was unclear whether SE, induced through systemic KA administration, is sufficient to induce epilepsy in C57 mice. SE was assessed over a 24h period as both the changes in power recorded through EEG activity and the behavioral seizures recorded by video camera and comprehensively scored offline (**Fig. 4A**). No seizures were ever observed in control mice (NaCl/VPA; N=3) during saline or VPA injections on day 1 (**Fig. 4A**) or during the 7 weeks of subsequent monitoring. For animals

enrolled in the ex-KA/VPA paradigm, an initial Racine stage 4/5 seizure was observed after 4-5 KA injections (total dose: 20-22.5 mg/kg), consistent with behavioral seizure experiments. All animals reached the criteria for behavioral SE (N=7/7). After 90 minutes of SE, VPA (300 mg/kg, i.p.) was administered to all mice, which immediately but transiently attenuated increases in power across all frequency bands (**Fig. 4A**). Similarly, animals enrolled in the ex-KA/NaCl paradigm displayed their first Racine stage 4/5 seizure after 3-6 KA injections (total dose: 17.5-25.0 mg/kg). Most ex-KA/NaCl animals (N=8/9) met the criteria for behavioral SE. Without VPA, increases in cortical power in ex-KA/NaCl animals self-terminated after a maximum length of two hours (**Fig. 4A**), suggesting the ex-KA/NaCl paradigm does not prolong SE for greater than 30 minutes when compared to the ex-KA/VPA paradigm. Offline scoring of behavioral seizure activity indicates animals in the KA/VPA and KA/NaCl cohorts typically displayed either three to seven distinct convulsive seizures (Racine stage 3-5), or a period of continuous SE defined by reared posture and forelimb clonus, typically lasting 30-50 minutes. Most notably, the minimal behavioral criteria of three Racine stage 4/5 seizures defining behavioral SE, did not correspond to pronounced increases in electrographic power (**Fig. 4A**, ex-KA/NaCl).

Over the course of 7 weeks, no spontaneous recurrent seizure was observed in either the ex-KA/VPA group (N=5/7 animals surviving 7 weeks), or the ex-KA/NaCl group (N=7/9 animals surviving 7 weeks).

An alternative low-dose KA administration paradigm produces robust behavioral and electrographic SE

We hypothesized the minor increase in EEG power coupled with the short length of electrographic SE in the ex-KA/VPA and ex-KA/NaCl conditions may have been insufficient to induce long-term, epileptogenic changes to the brain. Studies inducing later epilepsy in mice using pilocarpine indicate the length of SE, specifically lasting 2-3 hours, was best predictive of later spontaneous recurrent seizures (Mazzuferi et al., 2012). As such, we tested additional KA administration paradigms for their ability to prolong SE without significant mortality. Our first approach was to perform the ex-KA/NaCl paradigm as described previously, with the addition of a 2.5 mg/kg (i.p.) KA booster one hour after the first Racine stage 4/5 seizure was observed. Our second approach was to test another low-dose KA injection paradigm for C57BL6/J mice that was published previously. In this paradigm, described by Tse et al. (2014), C57 mice are administered KA (5 mg/kg, i.p.) until the first Racine stage 5 seizure is observed. We modified this paradigm to space injections by 20 minutes and included the booster concept described previously. Further, to reduce potential mortality, we administered a half dose (2.5 mg/kg, i.p.) of KA to animals displaying a Racine stage 3 seizure, if a stage 5 seizure was not observed before the time of next injection. In an initial study (N=3 mice/paradigm), we noted animals of the 5 mg/kg paradigm displayed Racine stage 5 seizures after the 6th injection (total dose: 30 mg/kg), while animals of the modified KA/NaCl paradigm displayed Racine stage 5 seizures after the 5th to 6th injection (total dose: 22.5-25.0 mg/kg). Importantly, the frequency and severity of behavioral seizures was observably greater for animals of the 5 mg/kg paradigm during the first hour of SE. Further, the period of behavioral seizure occurrence under either paradigm could be prolonged by the administration of a KA booster (2.5 mg/kg, i.p.) one

hour into SE without mortality. All initial study animals survived a one-week monitoring period without the administration of an anti-seizure drug. From these initially positive observations, we decided to adopt the 5 mg/kg paradigm, modified from Tse et al., for chronic EEG studies. Animals (N=14) were implanted with a cortical EEG and injected with low-dose KA according to the modified 5 mg/kg paradigm. Animals displayed a first Racine stage 5 seizure by the 4th to 5th injection (total dose: 17.5-25.0 mg/kg, including half-doses of KA).

Compared to the ex-KA/VPA or ex-KA/NaCl paradigms, electrographic SE was prolonged under the 5 mg/kg paradigm. Five of 14 animals displayed increases in electrographic power that did not appear to dissipate over an 18-24h time course; however, convulsive seizure activity did terminate after a maximum period of 4h in this subset of animals (example spectrum, **Fig. 4A**). The remaining nine animals displayed a pattern of electrographic SE that persisted for 2.5-3 h.

Spontaneous recurrent seizures observed under the 5mg/kg KA administration paradigm

Chronic monitoring over an 8-week period reveals unique correlations between SE, interictal spiking, and spontaneous recurrent seizures for animals enrolled in the 5 mg/kg paradigm. Of the animals exhibiting self-terminating electrographic SE over a 2.5-3.5h period (N=9/14), no animal displayed interictal spiking beyond a one-week period following SE or spontaneous seizures (**Fig. 5A**, denoted by a light blue bar beside the animal ID). For the five animals exhibiting continuously increased power over an 18-24h period (**Fig. 5A**, denoted by an orange bar beside the animal ID), two animals died within two weeks, before a full assessment of spontaneous seizure activity could be conducted. For the surviving three animals, two exhibited spontaneous recurrent seizures (**Fig. 5A**, red bars; **Fig. 5E**, representative trace) and all three animals displayed periods of interictal spiking that continued throughout the individual period of recording (**Fig. 5A**, dotted line; **Fig. 5C**, representative trace). All observed spontaneous seizures were fully generalized, tonic-clonic seizures meeting Racine stage 4/5 criteria. Subconvulsive seizures, meeting Racine stage 1/2 criteria (**Fig. 5A**, blue bar; **Fig. 5D**, representative trace) were infrequently observed within a 72h period following SE in a subset of animals displaying increased power over an 18-24h period.

Given the low prevalence of spontaneous seizure presentation, we searched for attributes of SE that may potentially predict later epilepsy development in the 5 mg/kg paradigm. During electrographic SE, the number of convulsive seizures and the seizure burden experienced by animals that developed epilepsy was notably greater (**Fig. 4B**). Animals not developing epilepsy experienced ten or less discrete convulsive seizures, while the few animals developing epilepsy experienced 30 or more discrete convulsive seizures during SE. Our observations suggest epilepsy development is possible in C57 mice using low-dose KA administration, but may require a seizure burden during SE that is greater than that typically produced through our investigated administration paradigms.

Absence of hypersynchronous activity during hippocampal depth electrode recordings

Our cortical electrode data could not rule out the possibility of subconvulsive hippocampal activity in the systemic low-dose kainate model. Electrographic features such as hippocampal paroxysmal discharges (HPDs) serve as a critical biomarker of hypersynchrony in the intrahippocampal kainate mouse model of mesial TLE (Riban et al., 2002). We implanted a small subset of mice with a hippocampal depth electrode targeted to the CA1 region of ventral hippocampus. Animals were administered saline to serve as controls (N=3) or kainate under the 5 mg/kg KA administration paradigm (N=3). Notably, during KA injections, we observed focal non-convulsive seizure activity and secondarily generalized seizures, suggesting the hippocampus contributes in seizure generation during SE (data not shown). All three kainate animals displayed at least three Racine stage 4/5 seizures during SE. However, over an 8-week monitoring period, no KA animal exhibited a spontaneous focal or secondarily generalized seizure in hippocampus. Features such as HPDs were additionally absent; however, we could detect interictal spikes in the hippocampus of similar character to the spiking observed through cortical recordings (**Fig. 5C**). At the conclusion of the study, depth electrode placement was histologically confirmed.

Discussion

Herein we describe a modified approach for the induction of SE in the genetically tractable C57BL/6J mouse. Our findings confirm a previous report (Tse et al., 2014) that low-dose, repeated KA administration serves as a useful paradigm for high rates of SE induction with limited mortality in C57BL/6J mice. Unique to our study, we demonstrate cell death can be readily observed in the hippocampus and limbic structures following repeated, low-dose KA administration in C57 mice. Our findings contrast an absence of cell death historically reported in C57 mice following a single bolus dose of KA. We additionally report reactive astrogliosis, and the expression of mGluR5 on hippocampal astrocytes in the sixth model of TLE to date. Finally, we observe spontaneous recurrent seizures can be induced in low-dose KA mice, but may be confined to animals experiencing a high seizure burden during SE.

Our purpose in characterizing low-dose, systemic KA paradigms is part of a larger effort to study mechanisms of epileptogenesis using the transgenic, knock-in, and knock-out technologies available on a C57BL/6 background. As such, our initial studies led us into the less explored area of repeated, low-dose KA insults, since responses to single or repeated doses of pilocarpine can result in high rates of mortality depending on vendor and substrain (Groticke et al., 2007; Muller et al., 2009; Müller et al., 2009; Oliveira et al., 2015). Given the high costs and time commitments associated with breeding transgenic animals, experimental epilepsy paradigms with poor rates of SE induction or high mortality would not be tenable. Initial paradigms focused on reaching a level of SE termed “behavioral SE,” defined by three or more convulsive seizures in a 90 minute period (Shibley and Smith, 2002). Under this conservative criteria for SE, 78.5% of animals (N=22/28) used in acute studies reached SE between the protracted KA/VPA, ex-KA/VPA, and ex-KA/NaCl paradigms. No animal died within a 7d study period. By contrast, single, bolus doses of KA typically result in mortality at a rate at or below 25% (Schauwecker, 2000; Schauwecker and Steward, 1997).

With the extensive evidence that a bolus dose of KA (s.c. or i.p.) does not produce cell death in wild-type C57 mice (McCord et al., 2008; Schauwecker, 2000, 2003, 2010, 2014; Schauwecker and Steward, 1997), despite the induction of SE, the consistency and extent of cell death observed in the KA/VPA and KA/NaCl paradigms was unexpected. Resistance to neurodegeneration following a bolus KA injection is attributable to genetic features of the C57 mouse (Schauwecker, 2010, 2014). Whether the pharmacokinetics of a repeated, low-dose KA insult are what circumvent the typical resistance to neurodegeneration following a bolus injection of KA remains to be determined. However, other variables beyond paradigm alone, such as kainate source, animal vendor/facility, and altitude, also differ between our studies. We were unable to test whether a single, bolus dose of KA (25 mg/kg, i.p.), common in the literature, would produce cell death in our hands due to the near-immediate lethality of the insult.

Both hallmark pathological features of TLE—cell death and astrogliosis in the temporal lobe—were present in the ex-KA/VPA and ex-KA/NaCl paradigms. Interestingly, administration of VPA 90 minutes into SE had insult modifying effects on the extent of cell death and astrogliosis. The pattern of cell death observed in ex-KA/VPA versus ex-KA/NaCl animals suggests VPA may confine excitotoxicity to the hippocampus. In the absence of VPA, excitotoxicity may secondarily impact limbic structures, such as thalamic nuclei and the amygdala in the ex-KA/NaCl condition. FJB-positive staining was not observed in non-convulsive animals treated with KA, suggesting a subconvulsive KA insult is not sufficient for excitotoxic cell death. However, the same non-convulsive animals did display hypertrophied astrocytes, suggesting KA can induce minor astrogliosis in the absence of convulsive seizure presentation. As shown in the wide-field images of cell death and astrogliosis in KA-seized animals, there was a reliable overlay of these two features within multiple regions of interest.

Understanding pathological changes in astrocytes during the development of epilepsy has gained attention with recent publications indicating that astrogliosis may be sufficient for seizure generation (Robel et al., 2015), or that metabolic pathways within the astrocyte can influence seizure activity (Sada et al., 2015). Prior research has also shown that activation of mGluR5, an astrocyte surface receptor, can serve as an upstream mediator of cell death following experimentally induced SE (Ding et al., 2007). Expression or functional activity of mGluR5 on astrocytes has been reported in five other models of epileptogenesis (Aronica et al., 2000; Ding et al., 2007; Ferraguti et al., 2001; Szokol et al., 2015; Ulas et al., 2000), with the low-dose, systemic KA mouse model representing the sixth model to date. The expression of mGluR5 on the mature astrocyte may be considered a feature of pathology, considering the expression of mGluR5 on astrocytes is limited to the first weeks of development in rodents (Morel et al., 2014; Sun et al., 2013).

Despite the emergence of pathological features following low-dose KA administration, the incidence and prevalence of spontaneous recurrent seizures in C57BL/6J mice was low during the 7-8 week time course of video EEG monitoring. Low rates of epilepsy may be attributable to a mild electrographic and behavioral SE presentation during KA administration. Mice displaying later epilepsy exhibited features correlated with epileptogenesis in rat, including longer SE durations (Gorter et al., 2001; van Vliet et al.,

2004), a higher seizure burden during SE, and interictal spiking patterns (Salami et al., 2014; Staley et al., 2011). The occurrence of a high seizure burden (20-30 convulsive motor seizures) or prolonged SE was uncommon under the described injection paradigms. High seizure burdens during SE are possible with the 5 mg/kg KA paradigm, but may require additional booster injections of KA for most animals. Determining the timing and appropriate dose of a booster injection would be facilitated through automated, real-time analyses of behavioral seizure activity and changes in EEG power. The ability for motion tracking software to identify behavioral seizures alongside existing algorithms to detect electrographic manifestations of seizures (Krook-Magnuson et al., 2013; Paz et al., 2012) could greatly aid the researcher in real-time seizure tracking during SE, especially across a large cohort. Further automated analyses could provide more objective criteria for repeated dosing regimens. Implementing these automated strategies may be especially important in the low-dose KA mouse model if a high seizure burden is necessary for epilepsy development.

Acknowledgements

We would like to thank Drs. Mike Bridge and Chris Rodesch of the Utah Cell Imaging Core for assistance with confocal microscopy and widefield imaging.

Funding: NIH RO1 NS078331 (JAW and KSW), NIH T32 NS076067 (ADU), NIH HHSN 271201100029C (HSW)

References

- Aronica E, van Vliet EA, Mayboroda OA, Troost D, da Silva FH, Gorter JA. Upregulation of metabotropic glutamate receptor subtype mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial temporal lobe epilepsy. *Eur J Neurosci.* 2000; 12:2333–2344. [PubMed: 10947812]
- Bankstahl M, Muller CJ, Wilk E, Schughart K, Loscher W. Generation and characterization of pilocarpine-sensitive C57BL/6 mice as a model of temporal lobe epilepsy. *Behav Brain Res.* 2012; 230:182–191. [PubMed: 22348894]
- Borges K, Gearing M, McDermott DL, Smith AB, Almonte AG, Wainer BH, Dingledine R. Neuronal and glial pathological changes during epileptogenesis in the mouse pilocarpine model. *Exp Neurol.* 2003; 182:21–34. [PubMed: 12821374]
- Chang CF, Huang HJ, Lee HC, Hung KC, Wu RT, Lin AM. Melatonin attenuates kainic acid-induced neurotoxicity in mouse hippocampus via inhibition of autophagy and alpha-synuclein aggregation. *J Pineal Res.* 2012; 52:312–321. [PubMed: 22212051]
- Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF, Coulter DA, Carmignoto G, Haydon PG. Enhanced astrocytic Ca²⁺ signals contribute to neuronal excitotoxicity after status epilepticus. *J Neurosci.* 2007; 27:10674–10684. [PubMed: 17913901]
- Ferraguti F, Corti C, Valerio E, Mion S, Xuereb J. Activated astrocytes in areas of kainate-induced neuronal injury upregulate the expression of the metabotropic glutamate receptors 2/3 and 5. *Experimental brain research.* 2001; 137:1–11. [PubMed: 11310162]
- Gorter JA, van Vliet EA, Aronica E. Status epilepticus, blood-brain barrier disruption, inflammation, and epileptogenesis. *Epilepsy Behav.* 2015; 49:13–16. [PubMed: 25958228]
- Gorter JA, van Vliet EA, Aronica E, Lopes da Silva FH. Progression of spontaneous seizures after status epilepticus is associated with mossy fibre sprouting and extensive bilateral loss of hilar parvalbumin and somatostatin-immunoreactive neurons. *Eur J Neurosci.* 2001; 13:657–669. [PubMed: 11207801]
- Groticke I, Hoffmann K, Loscher W. Behavioral alterations in the pilocarpine model of temporal lobe epilepsy in mice. *Exp Neurol.* 2007; 207:329–349. [PubMed: 17714705]

- Hellier JL, Patrylo PR, Buckmaster PS, Dudek FE. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. *Epilepsy Res.* 1998; 31:73–84. [PubMed: 9696302]
- Henshall DC, Engel T. P2X purinoceptors as a link between hyperexcitability and neuroinflammation in status epilepticus. *Epilepsy Behav.* 2015; 49:8–12. [PubMed: 25843343]
- Hesdorffer DC, Logroscino G, Cascino G, Annegers JF, Hauser WA. Risk of unprovoked seizure after acute symptomatic seizure: effect of status epilepticus. *Ann Neurol.* 1998; 44:908–912. [PubMed: 9851435]
- Hu RQ, Koh S, Torgerson T, Cole AJ. Neuronal stress and injury in C57/BL mice after systemic kainic acid administration. *Brain Res.* 1998; 810:229–240. [PubMed: 9813346]
- Krook-Magnuson E, Armstrong C, Oijala M, Soltesz I. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nat Commun.* 2013; 4:1376. [PubMed: 23340416]
- Lee JK, Jeong JW, Jang T, Lee GW, Han H, Kang JS, Kim IH. Decursin attenuates kainic acid-induced seizures in mice. *Neuroreport.* 2014; 25:1243–1249. [PubMed: 25171200]
- Mayer SA, Claassen J, Lokin J, Mendelsohn F, Dennis LJ, Fitzsimmons BF. Refractory status epilepticus: frequency, risk factors, and impact on outcome. *Arch Neurol.* 2002; 59:205–210. [PubMed: 11843690]
- Mazzuferi M, Kumar G, Rospo C, Kaminski RM. Rapid epileptogenesis in the mouse pilocarpine model: video-EEG, pharmacokinetic and histopathological characterization. *Experimental neurology.* 2012; 238:156–167. [PubMed: 22960187]
- McCord MC, Lorenzana A, Bloom CS, Chancer ZO, Schauwecker PE. Effect of age on kainate-induced seizure severity and cell death. *Neuroscience.* 2008; 154:1143–1153. [PubMed: 18479826]
- Morel L, Higashimori H, Tolman M, Yang Y. VGluT1+ neuronal glutamatergic signaling regulates postnatal developmental maturation of cortical protoplasmic astroglia. *J Neurosci.* 2014; 34:10950–10962. [PubMed: 25122895]
- Muller CJ, Groticke I, Hoffmann K, Schughart K, Loscher W. Differences in sensitivity to the convulsant pilocarpine in substrains and sublines of C57BL/6 mice. *Genes Brain Behav.* 2009; 8:481–492. [PubMed: 19493016]
- Müller, CJ., Gröticke, I., Bankstahl, M., Löscher, W. *Experimental Neurology.* Elsevier Inc.; 2009. Behavioral and cognitive alterations, spontaneous seizures, and neuropathology developing after a pilocarpine-induced status epilepticus in C57BL/6 mice; p. 284-297.
- Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci.* 2005; 25:7724–7733. [PubMed: 16120773]
- Oliveira CV, Grigoletto J, Funck VR, Ribeiro LR, Royes LF, Figuera MR, Furian AF, Oliveira MS. Evaluation of potential gender-related differences in behavioral and cognitive alterations following pilocarpine-induced status epilepticus in C57BL/6 mice. *Physiol Behav.* 2015; 143:142–150. [PubMed: 25749198]
- Paz JT, Davidson TJ, Frechette ES, Delord B, Parada I, Peng K, Deisseroth K, Huguenard JR. Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. *Nat Neurosci.* 2012:64–70. [PubMed: 23143518]
- Polli RS, Malheiros JM, Dos Santos R, Hamani C, Longo BM, Tannus A, Mello LE, Covolan L. Changes in Hippocampal Volume are Correlated with Cell Loss but Not with Seizure Frequency in Two Chronic Models of Temporal Lobe Epilepsy. *Front Neurol.* 2014; 5:111. [PubMed: 25071699]
- Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalography and clinical neurophysiology.* 1972; 32:281–294. [PubMed: 4110397]
- Riban V, Bouillere V, Pham-Le BT, Fritschy JM, Marescaux C, Depaulis A. Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. *Neuroscience.* 2002; 112:101–111. [PubMed: 12044475]
- Robel S, Buckingham SC, Boni JL, Campbell SL, Danbolt NC, Riedemann T, Sutor B, Sontheimer H. Reactive astrogliosis causes the development of spontaneous seizures. *J Neurosci.* 2015; 35:3330–3345. [PubMed: 25716834]

- Sada N, Lee S, Katsu T, Otsuki T, Inoue T. Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science*. 2015; 347:1362–1367. [PubMed: 25792327]
- Salami P, Levesque M, Benini R, Behr C, Gotman J, Avoli M. Dynamics of interictal spikes and high-frequency oscillations during epileptogenesis in temporal lobe epilepsy. *Neurobiol Dis*. 2014; 67:97–106. [PubMed: 24686305]
- Schauwecker PE. Seizure-induced neuronal death is associated with induction of c-Jun N-terminal kinase and is dependent on genetic background. *Brain Res*. 2000; 884:116–128. [PubMed: 11082493]
- Schauwecker PE. Genetic basis of kainate-induced excitotoxicity in mice: phenotypic modulation of seizure-induced cell death. *Epilepsy Res*. 2003; 55:201–210. [PubMed: 12972174]
- Schauwecker PE. Galanin receptor 1 deletion exacerbates hippocampal neuronal loss after systemic kainate administration in mice. *PLoS One*. 2010; 5:e15657. [PubMed: 21179451]
- Schauwecker PE. Susceptibility to seizure-induced excitotoxic cell death is regulated by an epistatic interaction between Chr 18 (Sicd1) and Chr 15 (Sicd2) loci in mice. *PLoS One*. 2014; 9:e110515. [PubMed: 25333963]
- Schauwecker PE, Steward O. Genetic determinants of susceptibility to excitotoxic cell death: implications for gene targeting approaches. *Proc Natl Acad Sci U S A*. 1997; 94:4103–4108. [PubMed: 9108112]
- Shibley H, Smith BN. Pilocarpine-induced status epilepticus results in mossy fiber sprouting and spontaneous seizures in C57BL/6 and CD-1 mice. *Epilepsy Res*. 2002; 49:109–120. [PubMed: 12049799]
- Staley KJ, White A, Dudek FE. Interictal spikes: harbingers or causes of epilepsy? *Neurosci Lett*. 2011; 497:247–250. [PubMed: 21458535]
- Stewart K-AA, Wilcox KS, Fujinami RS, White HS. Theiler's virus infection chronically alters seizure susceptibility. *Epilepsia*. 2009; 1418–1428. [PubMed: 20002148]
- Stewart KA, Wilcox KS, Fujinami RS, White HS. Development of postinfection epilepsy after Theiler's virus infection of C57BL/6 mice. *Journal of neuropathology and experimental neurology*. 2010; 69:1210–1219. [PubMed: 21107134]
- Sun W, McConnell E, Pare JF, Xu Q, Chen M, Peng W, Lovatt D, Han X, Smith Y, Nedergaard M. Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. *Science*. 2013; 339:197–200. [PubMed: 23307741]
- Szokol K, Heuser K, Tang W, Jensen V, Enger R, Bedner P, Steinhäuser C, Tauboll E, Ottersen OP, Nagelhus EA. Augmentation of Ca(2+) signaling in astrocytic endfeet in the latent phase of temporal lobe epilepsy. *Front Cell Neurosci*. 2015; 9:49. [PubMed: 25762896]
- Thom M. Hippocampal sclerosis: progress since Sommer. *Brain Pathol*. 2009; 19:565–572. [PubMed: 18761661]
- Thomson KE, White HS. A novel open-source drug-delivery system that allows for first-of-kind simulation of nonadherence to pharmacological interventions in animal disease models. *Journal of neuroscience methods*. 2014; 238:105–111. [PubMed: 25256646]
- Trinka E, Cock H, Hesdorffer D, Rossetti AO, Scheffer IE, Shinnar S, Shorvon S, Lowenstein DH. A definition and classification of status epilepticus - Report of the ILAE Task Force on Classification of Status Epilepticus. *Epilepsia*. 2015
- Trinka E, Hofler J, Zerbs A. Causes of status epilepticus. *Epilepsia* 53 Suppl. 2012; 4:127–138.
- Tse K, Puttachary S, Beamer E, Sills GJ, Thippeswamy T. Advantages of repeated low dose against single high dose of kainate in C57BL/6J mouse model of status epilepticus: behavioral and electroencephalographic studies. *PloS one*. 2014; 9:e96622. [PubMed: 24802808]
- Ulas J, Satou T, Ivins KJ, Kesslak JP, Cotman CW, Balazs R. Expression of metabotropic glutamate receptor 5 is increased in astrocytes after kainate-induced epileptic seizures. *Glia*. 2000; 30:352–361. [PubMed: 10797615]
- Umpierre AD, Remigio GJ, Dahle EJ, Bradford K, Alex AB, Smith MD, West PJ, White HS, Wilcox KS. Impaired cognitive ability and anxiety-like behavior following acute seizures in the Theiler's virus model of temporal lobe epilepsy. *Neurobiology of disease*. 2014; 64:98–106. [PubMed: 24412221]

- van Vliet EA, Aronica E, Tolner EA, Lopes da Silva FH, Gorter JA. Progression of temporal lobe epilepsy in the rat is associated with immunocytochemical changes in inhibitory interneurons in specific regions of the hippocampal formation. *Exp Neurol*. 2004; 187:367–379. [PubMed: 15144863]
- Vargas JR, Takahashi DK, Thomson KE, Wilcox KS. The expression of kainate receptor subunits in hippocampal astrocytes after experimentally induced status epilepticus. *Journal of neuropathology and experimental neurology*. 2013:919–932. [PubMed: 24042195]
- Wilcox KS, Gee JM, Gibbons MB, Tvrdik P, White JA. Altered structure and function of astrocytes following status epilepticus. *Epilepsy Behav*. 2015; 49:17–19. [PubMed: 26219575]
- Winawer MR, Gildersleeve SS, Phillips AG, Rabinowitz D, Palmer AA. Mapping a mouse limbic seizure susceptibility locus on chromosome 10. *Epilepsia*. 2011; 52:2076–2083. [PubMed: 21906048]
- Winawer MR, Klassen TL, Teed S, Shipman M, Leung EH, Palmer AA. A locus on mouse Ch10 influences susceptibility to limbic seizure severity: fine mapping and in silico candidate gene analysis. *Genes Brain Behav*. 2014; 13:341–349. [PubMed: 24373497]

HIGHLIGHTS

- We report that repeated, low-dose kainate administration paradigms produce hippocampal and limbic cell death in C57BL/6J mice, a feature historically absent from C57 mice injected with a bolus dose of kainate
- We observe repeated, low-dose kainate administration results in reactive astrogliosis and the expression of mGluR5 on astrocytes, which has been observed in parallel across multiple models of temporal lobe epilepsy
- We determined, for the first time, the chronic impact of a systemic kainate insult in C57BL/6J mice
- Rates of epilepsy following low-dose kainate administration were low or absent across three experimental status epilepticus induction paradigms, with epilepsy only developing in mice experiencing a high seizure burden during status epilepticus

Fig 1.

FluoroJade-B staining demonstrates cell death one week following low-dose KA administration. (A-B) Wide-field confocal images of DAPI (nuclei) and FJB staining (neurodegeneration) in a Control and ex-KA/NaCl coronal section. Multiple regions of interest (C-H) from the section displayed in (B) show positive FJB staining in the KA condition. (I) Cell death was quantified as the FJB-positive area by region across two coronal sections from control (N=5), ex-KA/VPA (N=5), and ex-KA/NaCl animals (N=5). FJB-positive staining was not observed in control animals (A) or non-seized animals (data

not shown). For ex-KA/VPA animals, FJB staining was elevated in the hilus, CA3, and CA1 regions of hippocampus. Without the seizure-attenuating effects of VPA, cell death in ex-KA/NaCl condition was not elevated in the hilus, but was elevated in all other regions analyzed, including the CA3 and CA1 cell body layers of hippocampus, the amygdala, dorsal thalamus, and ventral thalamus. Scale bar in (A): 500 μ m. *significant at $p<0.05$ and **significant at $p<0.01$, one-way ANOVA with Dunnett's post-hoc comparison to control.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2.

Prominent astroglial reactivity is observed in tissue from ex-KA/NaCl-treated animals. Astrocyte reactivity is quantified as the GFAP-positive area within a region of hippocampus or cortex. A wide-field image of GFAP and DAPI staining from a NaCl/VPA control section (**A**) and an ex-KA/NaCl section (**B**). (**C**) Raw GFAP staining in the hilus, in the cortical region superior to the hippocampus, and the *stratum oriens* and *stratum radiatum* surrounding the CA3 and CA1 cell body regions of hippocampus (dotted white lines demarcate the hilus and CA3 or CA1 cell body layer). Raw images illustrate the relatively low GFAP detection in control tissue, moderate GFAP detection in ex-KA/VPA tissue, and high GFAP detection in ex-KA/NaCl tissue (N=5/group; 2 sections/animal). (**D-G**) However, quantification of GFAP-positive area between control and KA-treated animals reveals GFAP-positive area is only elevated above control values in the ex-KA/NaCl condition within the hilus (**D**), surrounding the CA1 region (**E**), and surrounding the CA3 region (**F**). No differences in GFAP-positive area was detected in the overlying cortex (**G**). Scale bar in (**C**): 100μm.

*significant at $p < 0.05$, one-way ANOVA with Dunnett's post-hoc comparison to control.

Fig. 3.

Colocalization of mGluR5 and hippocampal astrocytes (GFAP) following low-dose KA treatment. Immunofluorescent detection of DAPI, mGluR5, and GFAP was performed in Control (A), ex-KA/NaCl (B), and ex-KA/VPA tissue (not shown). Channels are displayed individually (black and white), and merged for each image. Subfields (*stratum oriens* (SO), *stratum radiatum* (SR), and the CA1 pyramidal layer (CA1 S.P.)) are demarcated in the merged image. (C) Quantification of the colocalized area between GFAP and mGluR5 immunofluorescence suggests the expression of mGluR5 by hippocampal astrocytes is increased over control values in the regions surrounding the CA1 pyramidal layer for ex-KA/VPA tissue, and in the regions surrounding both the CA3 and CA1 pyramidal layers for ex-KA/NaCl tissue. The dotted black line in (C) represents the experimentally determined level colocalization from non-specific secondary antibody staining (primary omission slides). All colocalization values from KA-treated tissue are greater than the non-specific signal. *significant at $p < 0.05$ and **significant at $p < 0.01$, one-way ANOVA with Dunnett's post-hoc comparison to control.

Fig. 4. Electrographic activity and behavioral counts during low-dose KA SE. **(A)** Racine stage 1-5 behavioral seizure events are overlaid with corresponding changes to EEG power for a single animal from the control (NaCl/VPA), ex-KA/VPA, ex-KA/NaCl, or 5mg/kg KA conditions. The behavioral seizure counts and power spectra depicted for a 5mg/kg KA animal corresponds to an animal that later developed epilepsy. **(B)** We plotted the number of seizures and seizure burden for all animals enrolled in the 5 mg/kg KA paradigm and noted a

distinct increase in SE seizure activity for animals developing epilepsy. An “x” denotes an animal that died before 8-week study completion.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 5.

Interictal spiking, subconvulsive, and convulsive seizure occurrence in the 5 mg/kg paradigm. An 8-week history of epilepsy-related EEG events is charted in (A) following KA administration under the 5 mg/kg paradigm (N=14). For each animal (A1-A14), a light blue bar by the animal ID denotes a period of electrographic status lasting under 18h, while an orange bar denotes an 18-24h period of electrographic status. For each animal, incidents of interictal spiking (black dashed line), generalized seizures (red bars), and subconvulsive seizures (blue bars) are displayed. An example of the frequency and amplitude of interictal spiking is shown in (C). Subconvulsive seizures refer to any electrographic event meeting Racine stage 1-2 criteria. Such events were confined to a 72-h window after SE. An example trace is shown in (D). Generalized seizures refer to any electrographic event meeting Racine stage 3-5 criteria. In instances where more than one generalized seizure occurred in a 24-h period, a number above the red bar denotes the number of seizures clustered in that period. An example trace of a generalized seizure is shown in (E). A vertical line in (A) denotes disenrollment of an animal due to mortality. Scale bars in (B-E) correspond to 5mV (y-axis) and 5s (x-axis).

Table 1

Paradigms tested for acute pathological features

Paradigm	KA (m/k, i.p.)	VPA (m/k, i.p.)	N	Time to 1 st Seizure ¹	Total Dose ² (m/k, i.p.)	SE induction % (N)	Mortality ³ % (N)	Staining
High Dose	25	--	10	<5 min.	25	100% (10)	100% (10)	N/A
Protracted KA/VPA	5→2.5x	300	10	120-140	20-22.5	90% (9)	0% (0)	FJB, GFAP ⁴
Ex-KA/VPA	10→5→2.5x	300	6	60-120	20-22.5	83% (5)	0% (0)	FJB, GFAP, mGluR5
Ex-KA/NaCl	10→5→2.5x	--	10	80-140	22.5-25	60% (6)	0% (0)	FJB, GFAP, mGluR5

Kainate, KA; VPA, valproic acid; FJB, FluoroJade-B; GFAP, glial fibrillary acidic protein; mGluR5, metabotropic glutamate receptor 5;

'x' denotes repeated KA injections (i.p.) every 20 minutes until the first Racine stage 4/5 seizure was observed

¹ Seizure is a Racine stage 4/5 seizure

² Total Dose is the aggregate dose of KA administered before first seizure

³ Mortality encompasses a 7d period of observation

⁴ Analyses were performed but not reported due to the inefficiency of the paradigm

Table 2

Paradigms tested for chronic electrographic features

Paradigm	KA (m/k, i.p.)	VPA (m/k, i.p.)	N	Total Dose ¹ (mg/kg, i.p.)	SE induction % (N)	Mortality ² % (N)	SRS
Ex-KA/VPA	10→5→2.5x	300	7	20-22.5	100% (7)	29% (2)	0% (0)
Ex-KA/NaCl	10→5→2.5x	--	9	17.5-25.0	89% (8)	0% (0)	0% (0)
5 mg/kg	5x	--	14	20-30	71% (10)	21% (3)	14% (2)

Kainate, KA; VPA, valproic acid; SRS, spontaneous, recurrent seizures;

'x' denotes repeated KA injections (i.p.) every 20 minutes until the first Racine stage 4/5 seizure was observed

¹Total Dose is the aggregate dose of KA administered before first seizure

²Mortality encompasses a 7-8 week period of observation