Intravenous kainic acid induces status epilepticus and late onset seizures in mice

Nicolas D. Drysdale[1], **Elizabeth Matthews**[2], **Elizabeth Schuetz**[2], **Enhui Pan**[2], **James O. McNamara**[2,3,4]

[1]Duke University School of Medicine, Duke University Medical Center, Durham, NC 27710, USA

[2]Department of Neurobiology, Duke University Medical Center, Durham, NC 27710, USA

[3]Department of Medicine (Neurology), Duke University Medical Center, Durham, NC 27710, USA

[4]Department of Pharmacology and Molecular Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA

Abstract

We set out to establish a novel model of temporal lobe epilepsy (TLE) in a mouse. We sought to induce TLE through the injection of kainic acid (KA) into the tail vein with subsequent development of status epilepticus (SE). Using C57BL/6 mice, we implanted hippocampal EEG recording electrodes before or after injection of KA or phosphate buffered saline (PBS). Video and EEG analysis were conducted to evaluate for SE and development of recurrent seizures, the hallmark of TLE. All mice injected with KA developed SE while those who were injected with PBS did not. Of the animals injected with KA monitored for recurrent seizures following SE, 33% developed spontaneous recurrent seizures while those injected with PBS did not. Injection of KA through the tail vein of a mouse reliably and rapidly induces SE which remits spontaneously and leads to the development of TLE in a subset of mice.

Keywords

1. Introduction

Temporal lobe epilepsy (TLE) is a common and commonly devastating form of human epilepsy (Ropper et al., 2019). Animal models have been critical in elucidating mechanisms and developing treatments for this disease. One cause of TLE is an episode of prolonged seizures (status epilepticus [SE]) (Lewis et al., 2014; Liu et al., 2013). This has led to rodent

models in which experimental induction of SE is followed by emergence of TLE. Improved methods of generating mice with overexpression or deletion of a single gene as well as substituting a mutant gene identified in human disease have strengthened the rationale for development of mouse models in which SE promotes development of TLE.

Kainic acid is a chemoconvulsant that has been extensively utilized for models of status epilepticus and status epilepticus-induced TLE in rats and mice (Lévesque et al., 2016; Mouri et al., 2008; Rattka et al., 2013). These models have deployed diverse routes of administration of KA including °intracranial (including hippocampus and amygdala) injection as well as systemic routes. Thippeswamy and colleagues have implemented mouse models using intraperitoneal routes of administration of KA (Puttachary et al., 2015; Tse et al., 2014). A drawback of the intraperitoneal route is high inter-animal variability in the dosage and time required to elicit SE. The temporal control of KA delivery afforded by an IV bolus infusion together with prior studies of this route in rats (Lothman & Collins, 1981) led us to investigate SE and late onset TLE induced by IV KA administration in mice.

2. Methods

2.1. Animals

Male C57BL/6 mice purchased from Charles River Laboratories were used in these experiments. Mice were housed as a group and handled according to the NIH guide for the Care and Use of Laboratory Animals. All experiments were conducted under the approval of the Duke University Animal Care and Use Committee.

2.2. Surgical implantation of EEG electrodes and recording

A bipolar recording electrode was implanted in the left hippocampus using stereotaxic guidance (AP: −2.0mm, ML: −1.5mm, DV: −1.7mm) under isoflurane anesthesia in 8-week-old mice. A skull screw implanted overlying the right cerebellum served as ground electrode. For post-surgery cohorts, kainic acid injections were done 7 days following the implantation on 8-week old mice, and EEG recording was started immediately following injection. For the pre-surgery cohort, injections were done three days prior to implantations on 8-week old mice. The EEG recordings were started 7 days after implantation. EEG was recorded using head-mounted preamplifiers with 100X amplification and 1Hz high-pass filtering (Pinnacle Technologies). The electrical signal was digitized by 1401-Power2A A/D boards from Cambridge Electronic Design Ltd, and sampled at 500Hz with a 60Hz digital notch filter using Spike2 software. Video was captured using VMS Video Servers (GeoVision Inc) to multiplex IR cameras and compress the video files.

Spontaneous recurrent seizures were detected by review of EEG signals analyzed using version 9 of SPIKE 2 software (Cambridge Electronic Design, Ltd) with a custom algorithm written in our lab (Matthews et al., n.d.). The algorithm automatically detects periods when the power of the EEG in the gamma band (20–50Hz) exceeds the mean plus 3X standard deviation (mean + 3SD) of a user-defined baseline period for at least 10 seconds and is also followed by a post-ictal suppression period where the power in the gamma band falls at least 10X standard deviation below the mean. All detected seizure events were reviewed by

a trained user, and the video for 5 minutes before and after the detected event was examined for seizure scoring. The video and EEG features of events flagged as electrographic seizure were reviewed manually to verify the occurrence of a seizure.

2.3. Kainic Acid Infusion

Kainic Acid (Fisher Scientific) was dissolved in phosphate buffered saline (pH 7.4) at a concentration of one mg/ml and aliquoted, frozen, and stored at −80°degree C. On the day of infusion, an aliquot was removed from the freezer and allowed to come to room temperature prior to infusion; unused excess was discarded. Phosphate buffered saline (PBS) served as vehicle. Five units of heparin were added to every one ml of PBS and one mL of KA.

Animals were gently restrained (Broome Style Rodent Restrainer, Plas Labs) and either KA or PBS solutions infused into lateral tail vein via a 30 gauge needle at an infusion rate of 70 ul/min using a syringe pump (KdScientific). Following infusion, the mouse was placed in a clear cage within view of the camera for two-hour video EEG observation using a Grass system amplifier and recorder until the SE resolved. Animals that underwent surgical implantation after SE were video monitored for 2 hours without EEG. Animals that were subsequently used for long-term monitoring for seizure emergence were individually housed in the animal care facility, and monitored 24-hours per day for up to 40 days following SE.

Successful infusions were achieved in approximately 80% of mice. After a brief tutorial and practice, this success rate has been typical for each of five different individuals in the lab. Failed infusions due to inability to secure placement of needle in vein and/or leakage of fluid around the site of injection site were obvious; these animals were excluded from analyses.

2.4 Behavior Scoring of SE

Seizure behavior was assigned a score from zero to six according to a modified version of Racine scale introduced in 1972 (Phelan et al., 2015; Racine, 1972). Starting from the infusion, time was divided into epochs of five-minutes duration zero to five min., five to ten min. etc. The five-minute block was then assigned a score based on the highest category behavioral seizure during that time block. Unless specified otherwise, videos of the completed status epilepticus were analyzed for all cohorts, pre-surgery and post-surgery groups; these were reviewed for at least 60 minutes and required two successive time blocks with a score of zero before terminating review (Fig 1B). This strategy was utilized because the majority of animals resumed normal activity following two successive time blocks with a score of zero; it is possible that an occasional animal might have exhibited an additional seizure following this interval.

3. Results

3.1. Intravenous infusion of KA reliably induces SE

Initially, KA infusions (16 mg/kg) were administered to animals in which recording electrodes had been surgically implanted one week previously (Fig 1A, C, D). There were 8 animals in this cohort; 6 received KA infusions and 2 received PBS infusion (Table 1). Electrographic seizure activity was detected in hippocampal recordings within 6 minutes

of completing infusion (Fig 1D); these electrographic seizures correlated with behavioral seizures of Class 1 through 4 (Fig 1A) and were detected within 5–15 minutes. The electrographic seizure was characterized by the abrupt onset of rhythmic, high amplitude and high frequency spiking persisting for approximately 20 seconds at which time it terminated abruptly and was followed by suppression of background activity (Fig 1C). These electrographic seizures recurred over the ensuing 90 minutes accompanied by behavioral seizures ranging from Class 1 to 5. Overt behavioral seizures remitted spontaneously after approximately 90 minutes. A high mortality rate (4/6 mice, 67%) was observed among animals that underwent surgical implantation of electrodes prior to KA infusion. Because of this high mortality rate, a second group of animals was implanted and injected with a lower dose of KA (10–12mg/kg); this cohort consisted of 6 animals that received KA and 2 animals that received PBS. In this group, only 1/6 KA animals died during the period of SE. The remaining 7 mice were then followed for long-term monitoring to detect the emergence of spontaneous recurrent seizures (Table 1 & Fig 2A). Unfortunately files of videos of status epilepticus of these 5 KA animals could not be retrieved.

Because mortality induced by systemically administered kainic acid was reportedly diminished in animals without prior surgery (Balzekas et al., 2016), infusions were performed with an additional cohort of 34 animals without EEG recording electrodes (6 PBS, 28 KA); seizures induced by KA were monitored by video recording only. Fig 1B shows a representative subset of 18 animals that received KA infusions. Of the 28 animals that received KA (16mg/kg), only 5 died during or immediately following SE. In 12 of these animals (3 PBS, 9 KA), EEG recording electrodes were subsequently implanted 3 days following infusion, and the animals underwent long-term video-EEG to detect development of spontaneous recurrent seizures (Table 1 & Fig 2B). Mortality rates for the group that had electrodes implanted prior to KA infusion and received the higher dose of KA were significantly higher than for the group that had no surgery prior to KA infusion (Comparing 4/6 for pre-implantation vs 5/28 for post-implantation; Chi^2-statistic: 6.05, p=0.014, df = 1).

Review of video recordings revealed behavioral seizures with behavioral seizure class of 4 or greater in 18 of 28 animals. These behavioral seizures remitted spontaneously within 50–90 minutes following infusion; we can not exclude the possibility that electrographic seizure activity without behavioral correlates persisted. Despite receiving a complete infusion, heterogeneity of behavioral responses was observed (Fig 1 B). Typically, immediately following completion of KA infusion, the animal exhibited a paucity of movement (Class 1). In many animals, this was followed shortly (ranged from 2 to 15 minutes) by a Class 4 or 5 seizure. Class 2 seizures associated with head bobbing and Straub tail were often observed prior to the Class 4 or 5 seizure. Despite a complete infusion, some animals (1 of 18 animals shown in Fig 1B) never exhibited a behavioral seizure exceeding Class 3. In other animals, repetitive Class 4 and 5 seizures occurred; a subset of these animals died. In contrast to KA, infusions of PBS caused neither electrographic nor behavioral seizures in either cohort (data not shown).

Brains from a subset of animals were processed (6 KA animals, no electrodes implanted) for Fluro-jade staining 1 day after SE; all sub-regions of the hippocampus were examined

in sections containing dorsal, medial and ventral hippocampus. No evidence of Fluro-jade C positive staining was detected in any of the 6 animals (data not shown).

3.2. Mice Developed Spontaneous Recurrence of Seizures following SE

To determine whether KA-induced SE caused late onset spontaneous recurrent seizures (SRS), continuous video EEG recordings were conducted in a subset of mice that underwent implantation of electrodes before (n=7: 2 PBS, 5 KA) (Fig 2A) or after (n=12: 3PBS, 9 KA) (Fig 2B) infusion of KA or PBS. Recall that to avoid excessive mortality, the cohort which had surgery before KA infusion received a lower dose (10–12mg/kg). Despite the lower dose, the incidence of SRS was higher in the group that had surgery before KA infusion (4/5) than for the group that had KA infusion after surgery (2/9), although group sizes are small (Chi^2-statistics: 5.6, p<0.02, df=1). The latency to onset of the first SRS varied from from 9 to 30 days (Fig 2) with the pre-surgery infusion group having an average of 31 days, and the post-surgery infusion group having an average of 17.75 days (Fig. 2). The electrographic features of the SRS were similar to the pattern observed during SE, namely, abrupt onset of high frequency high amplitude spiking followed by postictal suppression (Fig 2C). Notably, behavioral features were characteristic of limbic seizures (Racine Class 1 to 3); no secondarily generalized clonic or tonic seizures (Racine Class 4 or 5) were observed. No seizures were detected in any of the six PBS infused mice from either cohort.

4. Discussion

The goal of these experiments was two-fold: a) to characterize status epilepticus induced by IV KA in adult mice; and b) to determine whether SE induced late onset spontaneous recurrent seizures. The following findings emerged: a) IV KA reliably induced the rapid onset of behavioral and electrographic seizures and the behavioral seizures terminated approximately 90 minutes after onset without treatment with antiseizure drugs; b) Continuous video EEG recordings over the ensuing several weeks revealed spontaneous recurrent seizures in ~ 30% percent of animals.

The IV KA model of SE described here exhibits disadvantages and advantages with respect to models utilizing intraperitoneal administration of KA described by Thippeswamy and colleagues (Puttachary et al., 2015; Tse et al., 2014). Disadvantages include the technical challenge of tail vein infusion. Also, if the experimental question requires a high proportion of animals to exhibit multiple spontaneous recurrent seizures, either repeated low dose KA or single high dose KA administered via an intraperitoneal route will deliver better results (Puttachary et al., 2015; Tse et al., 2014). Advantages of the IV route include improved control of the timing of KA access to the bloodstream and timing of seizure onset which can be useful for questions requiring temporal correlation of experimental measures with seizure activity. Also, the SE period remits spontaneously without need for treatment with anticonvulsants. Finally, experimental throughput is facilitated by a single infusion and remission without anticonvulsants. Notably, like results with intraperitoneal route of administration, acute seizure responses to intravenous KA were variable, ranging from severe status epilepticus with mortality to exclusively limbic seizures without Class 4 or 5 seizures.

The controlled and rapid access of KA to the bloodstream afforded by the IV route of administration enabled comparison of SE in our studies of mice with previous findings in rats. Striking differences emerged. IV infusion (15–30 sec) of a high dose of KA (12 mg/kg) into rats induced onset of electrographic seizure detected in hippocampal EEG recordings within a minute or two accompanied by arrest of movement, staring, and unresponsiveness to novel stimuli such as prodding or tapping, a pattern consistent with "limbic seizures"[9]. Mild limbic seizures persisted for roughly 30 minutes prior to emergence of intermittent wet dog shakes which persisted for another 30 minutes or so. Behavioral seizures including forelimb clonus, rearing and falling did not occur until 45–60 minutes following KA infusion. Like the rat, IV infusion of KA to a mouse induces onset of electrographic seizure in hippocampus within a minute or two. In stark contrast to the 45–60 minute latency between KA infusion and onset of seizures with forelimb clonus (Racine Class 4 or higher) in rat, IV KA induced behavioral seizures with forelimb clonus within five to fifteen minutes in the mouse. Whereas neuronal necrosis was evident in amygdala, pyriform cortex, and hippocampus one to six days following IV KA-induced SE in rats (Lothman and Collins, 1981) no cell death was detected with FJC staining in mice one day after SE. Two deoxyglucose studies of rats following IV KA revealed increased glucose utilization in hippocampus, subiculum, pyriform and entorhinal cortices and amygdala during the 30–45 minutes of "limbic seizures" and wet dog shakes prior to emergence of seizures with forelimb clonus and rearing. This prolonged period of mild "limbic seizures" correlating with selective engagement of limbic circuits in the rat was not observed in the mouse. The structural and functional mechanisms underlying the strikingly divergent responses to KA in these two species remain to be elucidated.

Another incidental finding of the present study is the high rate of mortality (67%) of the group of animals that had hippocampal bipolar electrodes implanted six days prior. In contrast, animals which underwent the SE initiation via tail-vein without prior surgery had lower mortality rates (18%). One possible explanation for the more severe SE and increased mortality in the group with prior implantation of recording electrodes is disruption of the blood-brain barrier, facilitating access of KA to the hippocampus, the likely site of seizure onset following systemic administration of KA (Mulle et al., 1998).

Funding:

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Highlights

- Injection of kainic acid in a mouse via the tail vein rapidly and reliably induces status epilepticus

- Systemic injection of kainic acid via the tail vein in mice shows heterogeneity in SE and seizure development

- Surgical implantations of EEG electrodes prior to kainic acid infusion increases mortality
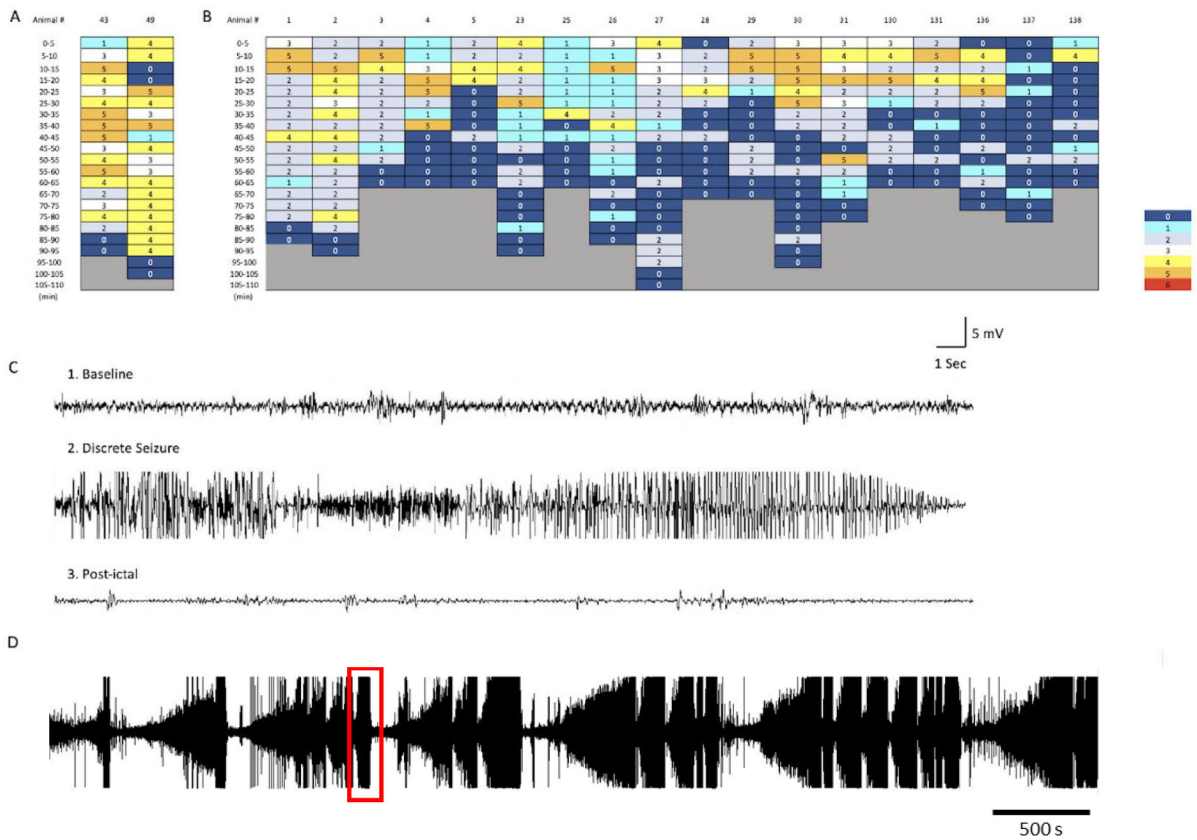
Figure 1. Tail vein infusion of KA lead to Status Epilepticus (SE) that spontaneously remits in Mice.

SE was considered remitted after 60 min and two consecutive scores of zero. Gray following behavior scores indicates end of SE and thus end of monitoring. (A) Racine behavior scores in five minute bins for mice who had surgery prior to infusion. (B) Racine behavior scores for mice who had infusion prior to surgery or no surgery. Color code for Racine scores is shown at far right. (C) A representative EEG demonstrating baseline, discrete seizure, and postictal period. (D) demonstrates the EEG from start of infusion to end of SE for the mouse from which the seizure example was taken. The red square indicates the isolated seizure followed by postictal suppression.
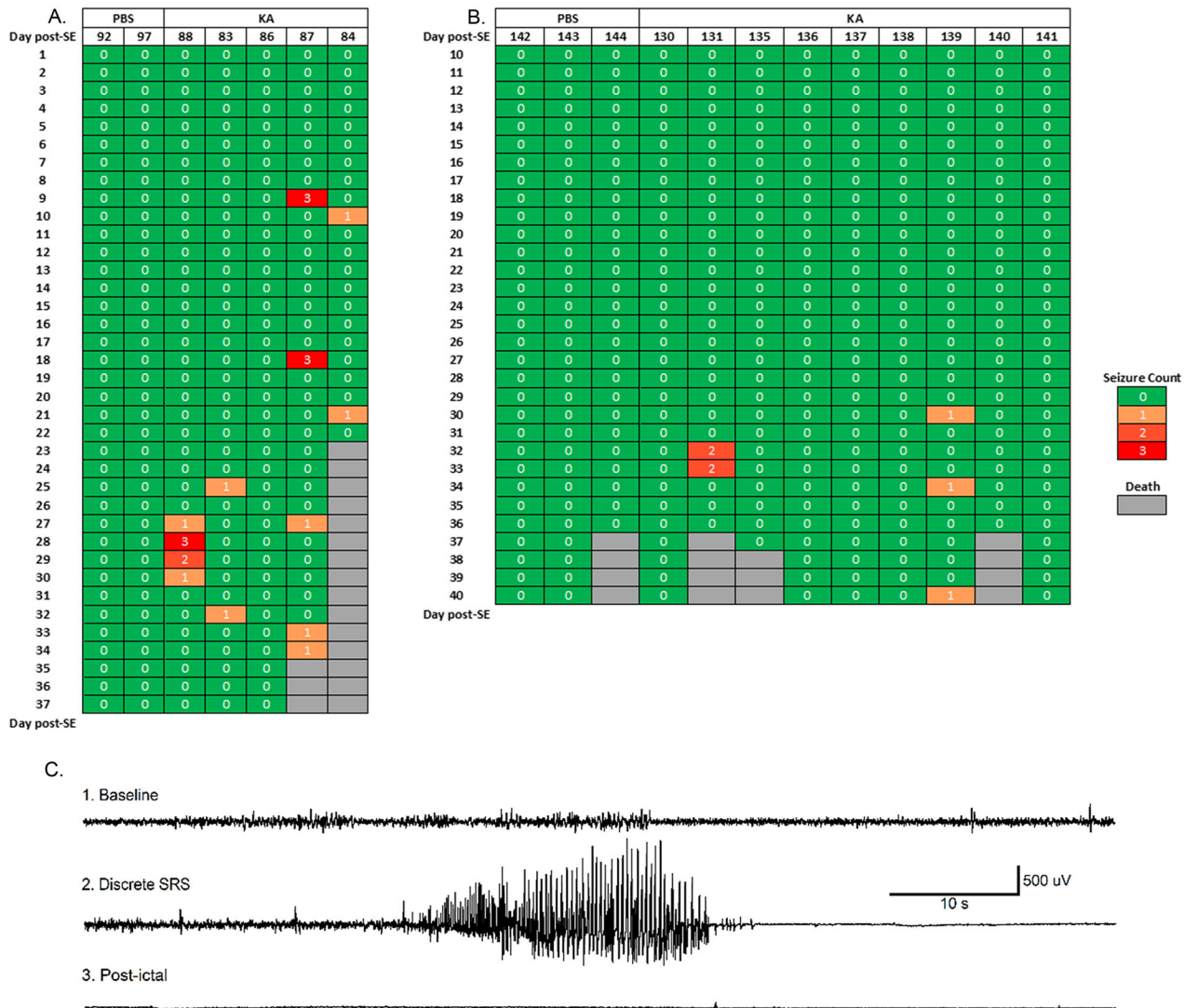
Figure 2. Tail vein infusion of KA leads to Spontaneous Recurrence of Seizures in Mice.
Number of seizures per day for mice throughout a long-term video and EEG monitoring period. Monitoring began 24 hours after SE for the group that had EEG electrodes implanted prior to KA infusion (A) or 10 days after SE for the group that had electrodes implanted after KA infusion (B). End of scoring for each animal marks time of death and thus end of the monitoring period. (C) A representative baseline, seizure, and post-ictal period from an SRS are shown.

Mice included in each cohort, their treatment, dosage, mortality, inclusion for long-term monitoring of SRS, and presentation of SRS

	Mouse ID	Treatment	Dose (mg/kg)	SE Mortality	Long-term monitoring	Developed SRS
Infusion following EEG surgery	43	KA	16	NO	NO	-
	48	KA	16	YES	NO	-
	49	KA	16	NO	NO	-
	45	KA	16	YES	NO	-
	38	KA	16	YES	NO	-
	98	KA	16	YES	NO	-
	99	PBS	-	NO	NO	-
	100	PBS	-	NO	NO	-
	83	KA	10	NO	YES	YES
	84	KA	10	NO	YES	YES
	85	KA	10	YES	DEAD	-
	86	KA	10	NO	YES	NO
	87	KA	12	NO	YES	YES
	88	KA	12	NO	YES	YES
	92	PBS	-	NO	YES	NO
	97	PBS	-	NO	YES	NO
Infusion prior to EEG surgery	130	KA	16	NO	YES	NO
	131	KA	16	NO	YES	YES
	132	KA	16	YES	DEAD	-
	133	KA	16	YES	DEAD	-
	135	KA	16	NO	YES	NO
	136	KA	16	NO	YES	NO
	137	KA	16	NO	YES	NO
	138	KA	16	NO	YES	NO
	139	KA	16	NO	YES	YES
	140	KA	16	NO	YES	NO
	141	KA	16	NO	YES	NO
	142	PBS	-	NO	YES	NO
	143	PBS	-	NO	YES	NO
	144	PBS	-	NO	YES	NO
Infusion only, no EEG surgery	1	KA	16	NO	NO	-
	2	KA	16	NO	NO	-
	3	KA	16	YES	NO	-
	4	KA	16	YES	NO	-
	5	KA	16	NO	NO	-

	Mouse ID	Treatment	Dose (mg/kg)	SE Mortality	Long-term monitoring	Developed SRS
	23	KA	16	NO	NO	-
	24	PBS	-	NO	NO	-
	25	KA	16	NO	NO	-
	26	KA	16	NO	NO	-
	27	KA	16	NO	NO	-
	28	KA	16	NO	NO	-
	29	KA	16	NO	NO	-
	30	KA	16	NO	NO	-
	31	KA	16	NO	NO	-
	32	PBS	-	NO	NO	-
	33	KA	16	NO	NO	-
	34	KA	16	NO	NO	-
	35	KA	16	YES	NO	-
	36	KA	16	NO	NO	-
	37	PBS	-	NO	NO	-