The intrahippocampal kainate mouse model of mesial temporal lobe epilepsy: Lack of electrographic seizure-like events in sham controls

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SUMMARY

Objective: There is an ongoing debate about definition of seizures in experimental models of acquired epilepsy and how important adequate sham controls are in this respect. For instance, several mouse and rat strains exhibit high-voltage rhythmic spike or spike-wave discharges in the cortical electroencephalogram (EEG), which has to be considered when using such strains for induction of epilepsy by status epilepticus, traumatic brain injury, or other means. Mice developing spontaneous recurrent nonconvulsive and convulsive seizures after intrahippocampal injection of kainate are increasingly being used as a model of mesial temporal lobe epilepsy. We performed a prospective study in which EEG alterations occurring in this model were compared with the EEGs in appropriate sham controls, using hippocampal electrodes and video-EEG monitoring.

Methods: Experiments with intrahippocampal kainate (or saline) injections started when mice were about 8 weeks of age. Continuous video-EEG recording via hippocampal electrodes was performed 6 weeks after surgery in kainate-injected mice and sham controls, that is, at an age of about 14 weeks. Three days of continuous video-EEG monitoring were compared between kainate-injected mice and experimental controls.

Results: As reported previously, kainate-injected mice exhibited two types of highly frequent electrographic seizures: high-voltage sharp waves, which were often monomorphic, and polymorphic hippocampal paroxysmal discharges. In addition, generalized convulsive clinical seizures were infrequently observed. None of these electrographic or electroclinical seizures were observed in sham controls. The only infrequently observed EEG abnormalities in sham controls were isolated spikes or spike clusters, which were also recorded in epileptic mice.

Significance: This study rigorously demonstrates, by explicit comparison with the EEGs of sham controls, that the nonconvulsive paroxysmal events observed in this model are consequences of the induced epilepsy and not features of the EEG expected to be seen in some experimental control mice or unintentionally induced by surgical procedures.

KEY WORDS: EEG, Oscillations, High-voltage rhythmic spikes, Spike-wave discharges, Hippocampus.
**Key Points**

- Inherent or acquired EEG abnormalities in experimental controls may present a bias for interpretation of paroxysmal EEG alterations in epilepsy models.
- We compared EEG alterations in mice 6 weeks following intrahippocampal kainate injection with EEG recordings in sham controls.
- Frequent electrographic seizures were recorded in kainate-treated mice, whereas such seizures were not observed in controls.
- The only EEG alteration in controls were infrequent spikes or spike clusters, which were also observed in epileptic mice.
- These data substantiate that the seizure-like EEG events in mice of the kainate model are electrographic seizures and do not occur in controls.

Focal seizures in this model are characterized by high-voltage sharp waves (HVSWs) and hippocampal paroxysmal discharges (HPDs), which are only recorded close to the kainate focus in the ipsilateral hippocampus and which may or may not be associated with mild behavioral changes. These nonconvulsive focal seizures (termed electrographic seizures in the following) occur spontaneously about 20–60 times per hour. HVSWs are often monomorphic, and it has been discussed whether they represent ictal or interictal events.

However, most studies using the intrahippocampal kainate mouse model of TLE did not include sham controls in electroencephalogram (EEG) monitoring, so it cannot be excluded with enough certainty that at least part of the paroxysmal EEG activity, for example, the HVSWs recorded in this model, represents repetitive, synchronous hyperactivity or oscillatory activity that is also present in “normal” controls and that is distinct from simple or complex partial seizures resulting from the kainate focus in the hippocampus. Furthermore, paroxysmal EEG activity may be a consequence of the lesion and associated blood-brain barrier disruption and neuroinflammation produced by a depth EEG electrode, particularly if implanted in limbic regions such as the hippocampus.

Before concluding that paroxysmal EEG alterations were produced by a lesion (kainate, electrode, or both), it is also important to consider that experimental controls were produced from several otherwise “normal” mouse strains exhibit frequent 6- to 8-Hz spike-wave discharges (SWDs) in the EEG, which may impede the interpretation of acquired EEG alterations in the intrahippocampal kainate model.

This prompted us to perform a prospective study in which EEG alterations occurring in this model were compared with the EEGs in appropriate sham controls, using hippocampal electrodes and video-EEG monitoring. For this study we chose male mice of the NMRI (Naval Medical Research Institute) outbred strain, a general-purpose stock in many fields of research including pharmacology, which was previously used for this model by our group and others. We expect that our study adds to the current discussion about how to best define a “seizure” in experimental models of acquired epilepsy and how careful study of control animals can clarify this issue.

The few previous studies on the intrahippocampal kainate mouse model in which sham controls were included used only a few animals for this purpose whereas we chose a relatively large group size for our study, thus allowing statistical comparisons with kainate-injected mice.

**Materials and Methods**

**Animals**

Outbred male NMRI mice, which originated from a colony of Swiss mice and which are extensively used as a general-purpose stock in many fields of research including pharmacology, were obtained from Charles River (Sulzfeld, Germany) at an age of 4–7 weeks (body weight 20–36 g). Mice were adapted to the laboratory conditions for 1–2 weeks before being used in experiments so that all mice were midadolescent age at time of kainate injection. Animals were housed under controlled conditions (ambient temperature 22–24°C, humidity 30–50%, lights on from 6:00 AM to 6:00 PM). Food (Altromin 1324 standard diet; Altromin, Lage, Germany) and water were freely available.

Experiments were performed according to the European Union (EU) council directive 2010/63/EU and the German Law on Animal Protection (“Tierschutzgesetz”). Ethical approval for the study was granted by an ethical committee (according to §15 of the Tierschutzgesetz) and the government agency (Lower Saxony State Office for Consumer Protection and Food Safety; LAVES) responsible for approval of animal experiments in Lower Saxony (reference numbers for this project: 09/1769 and 14/1659). All efforts were made to minimize both the suffering and the number of animals.

**Intrahippocampal kainate model in mice**

In this model, status epilepticus (SE) is induced by unilateral injection of kainate into the dorsal hippocampus. For this purpose, mice were anesthetized with chloral hydrate (500 mg/kg i.p.) and kainate (0.21 μg in 50 nl saline; i.e., 1 nm), which was obtained from Sigma-Aldrich (Steinheim, Germany), was stereotaxically injected into either the right CA1 (n = 9) or the right dentate gyrus (DG; n = 6) of the dorsal hippocampus (see Fig. 1). Kainate was slowly injected over 60 s with a 0.5 μl microsyringe. In preliminary experiments in groups of 6 mice, stereotaxic coordinates (according to Paxinos and Franklin) were determined by histological verification of injection site in...
CA1 or DG. These coordinates were then used for the experiments described in this study: CA1, AP, −2.10 mm; L, −1.60 mm; V, −2.30 mm; DG, AP, −2.10 mm; L, −1.60 mm; V, −1.8 mm. After injection of kainate, the needle of the syringe was maintained in situ for an additional 2 min to limit reflux along the injection track. For EEG recordings, the animals were immediately implanted with bipolar electrodes aimed at the site of kainate injection in the ipsilateral CA1, using the same coordinates as for kainate injection (see Gröttick et al.3). During all surgical procedures and for about 1 h thereafter, mice were kept on a warming pad to avoid hypothermia.

Sham controls
For sham controls, all procedures were exactly the same as for the kainate-injected mice except that either nothing (n = 6) or saline (n = 6) was injected instead of kainate (Fig. 1). In animals with saline injections, all details of injection (volume, speed) were the same as for kainate injection described above.

Video/EEG recording
After surgery, EEG/video monitoring was used to verify the limbic, predominantly nonconvulsive SE induced by kainate. Starting 6 weeks after SE, that is, at a time when the majority of kainate-injected mice exhibited spontaneous recurrent seizures,8,17 video-EEG monitoring was used to compare the occurrence of seizures or other EEG abnormalities in kainate-injected versus sham control mice (Fig. 1).

For EEG recording, mice were connected via a flexible cable to a system consisting of eight 1-channel bioamplifiers (ADInstruments Ltd., Sydney, NSW, Australia) and an analog-digital converter (PowerLab 8/30 ML870 or PowerLab 4/35 PL3504/P; ADInstruments). The data were recorded (sampling rate 200 Hz, time constant 0.1 s, low-pass filter of >60 Hz, 50-Hz notch filter) and analyzed with LabChart 6 or 8 for Windows software (ADInstruments). The EEG recording was directly linked to simultaneous digital video recording of 4–8 per system using either one high-resolution infrared camera for up to 8 mice (NYCTO Vision; CaS Business Services, Wunstorf, Germany) or four infrared board cameras (Sony) for 4 mice merged by one video quad processor (Monarcor TVSP-44COL). For video/EEG monitoring, mice were housed singly in clear plexiglass cages (one per cage). For monitoring during the dark phase, infrared LEDs were mounted above the cages.

Three days of continuous video-EEG monitoring were compared between kainate-injected mice and sham controls (Fig. 1). All EEGs were visually examined for abnormal electrographic activity. Three days was considered sufficient because the frequency of the electrographic seizures (HVSWs and HPDs) is so high in epileptic mice of the intrahippocampal kainate model8,17 that even recording for a few hours would have been sufficient for the goal of the present study.

Statistics
Fisher’s exact test was used to determine significance of differences in seizure occurrence in kainate-treated versus sham control mice. A p ≤ 0.05 was considered significant.

Results
Analysis of epileptic EEG activity
HVSWs, HPDs, and interictal EEG activity were defined as described recently.8 Typical examples are shown in Fig. 2. As recently arbitrarily defined by us for the mouse strain and model characteristics used in our studies,17 HVSWs are characterized by sharp waves with high amplitude of at least three times the EEG baseline, have a duration of at least 5 s, occur at a frequency of at least 2 Hz, and have an interevent interval of at least 3 s. During the interevent
interval, there is either no epileptic EEG activity or isolated spikes or spike trains with an amplitude of <3 times baseline, which was considered as interictal activity. HVSWs can either show no clear evolution or some evolution in frequency or pattern. HPDs always exhibit evolution in morphology and frequency and are often longer (>20 s) than typical HVSWs. They typically start with large-amplitude HVSWs, followed by a train of lower-amplitude spikes (≥2 times baseline) of at least 5 s of increased frequency (≥5 Hz). Similar to HVSWs, during interevent intervals (at

Figure 2.
Representative examples of EEG alterations observed in sham controls and mice with intrahippocampal kainate injections. All recordings were performed 6 weeks after kainate injection or sham preparation via a depth electrode implanted in the kainate focus in either CA1 or DG (see Fig. 1). (A) Normal baseline activity without any paroxysmal alterations in a sham control mouse. This activity was predominantly recorded in sham control mice. Higher magnification showed the typical theta rhythm (rhythmic slow wave activity, 6–9 Hz) occurring in hippocampal recordings of mice. (B) Isolated spikes in the hippocampal EEG of a sham control mouse. This activity was also seen in kainate-treated mice. (C) Spike cluster in the hippocampal EEG of a sham control mouse. This activity was also seen in kainate-treated mice. (D) An electrographic seizure of the HVSW type in an epileptic mouse of the kainate group. Such activity was never observed in sham controls (see Table 1). (E) An electrographic seizure of the HPD type in an epileptic mouse of the kainate group. Such activity was never observed in sham controls (see Table 1). (F) EEG alterations during a clinical (generalized convulsive) seizure in an epileptic mouse of the kainate group. Such EEG or clinical activity was never observed in sham controls (see Table 1).

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least 3 s) no epileptic EEG activity or isolated spikes or spike trains with an amplitude of <2 times baseline was observed, which was considered as interictal activity. In addition to these typical HPDs, a second type was observed, which looked like a mixed event starting with HPD-like addition to these typical HPDs, a second type was observed, which was considered as interictal activity. In spike trains with an amplitude of < 3 s) no epileptic EEG activity or isolated spikes or spike clusters, whereas electrographic seizures (HVSWs, HPDs) frequently occurred in kainate-injected mice (see Fig. 2).

### EEG alterations in the two groups of epileptic mice

The two kainate groups (CA1 vs. DG) were indistinguishable in terms of seizure characteristics (Table 1). Both groups exhibited interictal spikes or spike clusters and frequent electrographic seizures (monomorphic or polymorphic HVSWs and polymorphic HPDs) in the EEG recorded from the kainate focus, as shown in Fig. 2. In addition, generalized convulsive seizures were infrequently observed in several mice over the 3 days of continuous video-EEG monitoring used for these comparisons. Additional observations demonstrated that such clinical seizures occurred in the majority of kainate-treated mice.

### EEG alterations in experimental controls

In the 12 sham controls, the hippocampal EEG showed normal baseline activity, which was only occasionally interrupted by isolated spikes or spike clusters, as shown in Fig. 2. The spikes or spike clusters resembled in their morphology those seen in kainate-treated mice, but spike clusters occurred more frequently in epileptic mice (Table 1). In contrast to mice with intrahippocampal kainate, electrographic (HVSWs, HPDs) or electroclinical seizures were not observed in sham controls (Table 1). This difference to epileptic mice was highly significant (Table 1), demonstrating that the study was sufficiently powered to detect significant intergroup differences.

### Discussion

Laboratory mouse and rat strains often serve as “normal” controls for neurological and other studies, but as shown for many phenotypes, they are not necessarily wild-type, because they segregate natural genetic variants that either predispose to disease or cause it outright. For instance, in several mouse strains, occasional or frequent periods of bilateral synchronous 6- to 8-Hz SWD activity occurs in the cortical EEG. This activity is associated with behavioral arrest and is suppressed by the antiabsence drug ethosuximide, similar to epileptic absence-like activity. In the normal mouse hippocampus, theta (6–9 Hz) and gamma (40–100 Hz) oscillations are present during exploration and rapid eye movement sleep. During immobility, consummatory behaviors, and slow-wave sleep, sharp waves are recorded in CA1 of the hippocampus. Event-related oscillations, that is, rhythmic changes that are evoked by sensory and/or cognitive processes and that influence the dynamics of the EEG, can be recorded from cortical sites in experimental control mice in response to auditory stimulation. The typical HVSWs and HPDs recorded from CA1 or DG in epileptic NMRI mice of the intrahippocampal kainate model in the present study did not resemble any of these oscillations and did not occur in sham controls. Also, 6- to 8-Hz SWDs were observed neither in the epileptic mice nor in sham controls of the NMRI strain under the conditions of the present study. In rats, short-lasting EEG episodes of medium-voltage 5- to 9-Hz (mean = 6 Hz) cortical oscillations, which were distinguishable from sleep spindles in internal frequency,
duration, morphology, and moment of occurrence, were described as normal physiological EEG activity.\textsuperscript{29,30} Extensive analysis has shown that 5- to 9-Hz oscillations alone cannot lead to seizure activity, but genetic manipulations (like the ones resulting from successive inbreeding) are required to make seizure-like SWD activity emerge.\textsuperscript{29} As observed in mouse strains, several outbred and inbred rat strains exhibit bilaterally symmetrical SWDs (8–11 Hz; sometimes also termed high-voltage rhythmic spike [HVRS] discharges, high-voltage cortical oscillations, or electrocortical polyspiking)\textsuperscript{31–34} in the cortical EEG, which are associated by sudden arrest of ongoing behavior (immobility) with occasional facial/whisker twitching, and which can be suppressed by ethosuximide, thus behaving as an absence-like seizure activity.\textsuperscript{35–38}

In addition to low-frequency 5- to 9-Hz oscillations or SWDs, high-frequency oscillations (HFOs) at frequencies in the range of 30–60 Hz occur in several cortical and subcortical brain areas of rodents.\textsuperscript{39} For instance, large-amplitude local field potentials (“sharp waves”; SPWs) occur irregularly in the hippocampal CA1 stratum radiatum of control rats when the animal has minimal or no interaction with its environment, such as during immobility, consummatory behaviors, or slow wave sleep.\textsuperscript{39} SPWs reflect the depolarization of the apical dendrites of CA1 and CA3 pyramidal cells due to the synchronous bursting of CA3 pyramidal cells. Such physiological population patterns in the brain are characterized by their strict bounds of both duration and synchrony.\textsuperscript{39} This organization is in sharp contrast to epileptic patterns in which scale-free behavior dominates, and, therefore, event magnitudes vary several orders of magnitude.\textsuperscript{39–41} The distinction between endogenous and experimentally induced HFOs (or any other type of oscillation in the cortical or subcortical EEG) can be made only by directly comparing epileptic animals with sham controls. In addition, in both rats and mice, the lesion associated with implantation of a depth electrode into the limbic system may induce proepileptogenic brain alterations, including blood-brain barrier disruption, chronic inflammation, decreases in seizure threshold, and epileptiform discharges in the hippocampus.\textsuperscript{9–11} so it is important to include adequate sham controls when studying video-EEG alterations in rodent models of acquired epilepsy such as the intrahippocampal kainate model.

In one of the initial studies on the intrahippocampal kainate mouse model of TLE, which was performed in male Swiss outbred mice, Riban et al.\textsuperscript{2} included 4 control mice in which saline was injected into the hippocampus instead of kainate. When EEG recordings were performed during the resting phase of the mice, typically from 16.00 to 19.00 h, over 2 months following kainate or saline injection, mice injected with saline showed a typical desynchronized activity in the hippocampus when the animals were awake. In addition, theta rhythm (5–7 Hz) was often observed on the hippocampal derivation concomitantly with active sniffing, exploration, and sleep. Occasionally, isolated low-voltage spikes (800–1,100 μV) could be observed in sham controls, but the typical HVSWs and HPDs occurring in kainate-injected mice were not observed in sham controls.\textsuperscript{2} Likewise, no paroxysmal activity was recorded in the cerebral cortex of control mice. In a subsequent study in Swiss mice, these observations were confirmed in a group of five saline-injected sham controls, showing that theta activity dominated the overall EEG activity and abnormal activity was not recorded from saline-injected animals.\textsuperscript{23} In a study in male C57BL/6 inbred mice, in which 5 mice were injected with saline instead of kainate, also only normal theta rhythm was observed in the hippocampal recordings.\textsuperscript{24} Furthermore, no paroxysmal activity was observed in the cortex.

In apparent contrast, when female FVB/N inbred mice were used for the kainate model, short seizure-like events (SLEs; mostly <10 s) resembling HVSWs were frequently observed in the hippocampal EEG in all 7 sham controls.\textsuperscript{7} Average frequency of such SLEs was 0.65/h (compared to 116/h in 6 kainate-treated epileptic mice; p < 0.0001) except for 1 sham control mouse, in which average SLE frequency was 22/h. Long (>20 s) SLEs (mostly HVSWs) were observed only in kainate-treated epileptic mice (average frequency 10.8 ± 1.9 per h). The lack of SLEs in sham controls of previous and the present experiments in other mouse strains may indicate mouse strain differences.

In the present study in male NMRI mice, most of the 12 sham controls exhibited normal hippocampal EEG activity without any indication of electrographic seizures (HPDs, HVSWs) as observed in kainate-injected mice. In all 12 sham controls, isolated spikes were occasionally observed. In addition, 3/12 mice exhibited infrequent spike clusters that differed in morphology from the electrographic seizures observed in the kainate-injected mice. Whether these few apparent EEG abnormalities observed in sham controls are a result of the lesion produced by the hippocampal electrode or are due to other reasons cannot be determined at present, but our study clearly substantiates previous reports with lower group size of sham controls in other mouse strains,\textsuperscript{22,24} demonstrating that the typical electrographic seizures (HVSWs, HPDs) observed in epileptic mice of the kainate model do not occur in sham controls. In this respect it is also important to note that secondarily generalized convulsive seizures were observed in most kainate-treated mice, but never in sham controls. Furthermore, inherent SWDs were not observed in the EEGs of sham controls, which is in line with previous reports that spontaneous SWDs (or other types of nonconvulsive seizures) do not occur in most inbred and outbred mouse strains, including the strain used in the present study,\textsuperscript{11} but may occur in some inbred strains such as DBA/2, C3H/HeJ, and A/J.\textsuperscript{12} However, the possibility that epilepsies may occur spontaneously in naive control rodents cannot be excluded for strains that have not been reported to exhibit epileptic EEG
alternations before, so sham controls should be included in experiments on any new batch of mice delivered to the laboratory from a vendor, independently of whether outbred or inbred strains are used. For multipurpose mouse outbred strains such as NMRI, Swiss, or CD-1, it should be considered that such randomly outbred strains are known to be genetically heterogeneous populations with a high intrastrain variation. Genetic divergence between outbred subpopulations may arise from a number of processes, including mutation, natural selection, unconscious selection, and random genetic drift. Intrastrain differences may be an important reason for discrepancies between studies from different laboratories. Furthermore, apart from genetics, intrastrain and interstrain differences in SE models can also be due to the environmental factors of where the animals are bred and maintained.

We have recently reported that based on clinical criteria of nonconvulsive seizures and their pharmacology, both HVSWs and HPDs occurring in the chronic phase after kainate-induced SE in NMRI mice can be considered electrographic seizures. In male NMRI mice, high doses of carbamazepine and low doses of diazepam and phenobarbital rapidly suppressed both HVSWs and HPDs. These data demonstrated that focal electrographic seizures in the intrahippocampal kainate mouse model are less resistant than previously thought and that both mouse strain and the criteria chosen for definition of EEG seizures determine whether such seizures are drug-resistant or -responsive.

An apparent contrast between our studies in NMRI mice and several previous studies by other groups in Swiss and C57BL/6 mice is that we did not observe any clear behavioral correlates of epileptic EEG activity (HVSWs and HPDs) in epileptic mice of the kainate model, whereas previous studies reported that HPDs were often associated with behavioral arrest, head nodding, or stereotyped behavior, such as exploration or grooming, which may be so subtle that they are not easily recognized or differentiated from normal locomotor activity of mice. Similar to our studies, Maroso et al. using C57BL/6 mice for the intrahippocampal kainate model, reported that HPDs and HVSWs were subclinical paroxystic events, resembling the subclinical seizures seen in humans with TLE during intracranial monitoring, and differing from interictal activity. In patients, mesial temporal lobe seizures that lack a scalp ictal correlate are often electrographic seizures that occur without obvious clinical manifestations. An intracranial depth electrode study in patients with TLE showed that 80% of seizures that arose focally and that remained focal within the mesial temporal lobe were not associated with any clinical symptoms. Indeed, seizures with clinical symptoms are often only a small proportion of all abnormal electrographic activity in the brain, which includes subclinical seizures, interictal spikes, bursts, and high-frequency oscillations. This spectrum of subclinical, clinical, and interictal epileptic activities occurring in TLE is nicely reproduced by the intrahippocampal kainate model in mice.

In conclusion, our study substantiates that appropriate age-matched sham controls are essential in unambiguously determining whether paroxysmal EEG alterations occurring in a model of acquired epilepsy are a consequence of the induced epilepsy or due to inherent or electrode-induced EEG abnormalities also present in the controls. An International League Against Epilepsy/American Epilepsy Society Translational Research Task Force is currently working on the harmonization of video-EEG interpretation and analysis in rodents, both sham control and epileptic animals, with the purpose of increasing the translational value of animal models. The present study may be helpful for this important purpose.

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**DISCLOSURE**

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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