A face-to-face comparison of the intra-amygdala and intrahippocampal kainate mouse models of mesial temporal lobe epilepsy and their utility for testing novel therapies

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Abstract
Objective: Intracranial (intrahippocampal or intra-amygdala) administration of kainate in rodents leads to spatially restricted brain injury and development of focal epilepsy with characteristics that resemble mesial temporal lobe epilepsy. Such rodent models are used both in the search for more effective antiseizure drugs (ASDs) and in the development of antiepileptogenic strategies. However, it is not clear which of the models is best suited for testing different types of epilepsy therapies.

Methods: In the present study, we performed a face-to-face comparison of the intra-amygdala kainate (IAK) and intrahippocampal kainate (IHK) mouse models using the same mouse inbred strain (C57BL/6). For comparison, some experiments were performed in mouse outbred strains.

Results: Intra-amygdala kainate injection led to more severe status epilepticus and higher mortality than intrahippocampal injection. In male C57BL/6 mice, the latent period to spontaneous recurrent seizures (SRSs) was short or absent in both models, whereas a significantly longer latent period was determined in NMRI and CD-1 outbred mice. When SRSs were recorded from the ipsilateral hippocampus, relatively frequent electroclinical seizures were determined in the IAK model, whereas only infrequent electroclinical seizures but extremely frequent focal electrographic seizures were determined in the IHK model. As a consequence of the differences in SRS frequency, prolonged video-electroencephalographic monitoring and drug administration were needed for testing efficacy of the benchmark ASD carbamazepine in the IAK model, whereas acute drug testing was possible in the IHK model. In both models, carbamazepine was only effective at high doses, indicating ASD resistance to this benchmark drug.

Significance: We found a variety of significant differences between the IAK and IHK models, which are important when deciding which of these models is best suited for studies on novel epilepsy therapies. The IAK model appears particularly interesting for studies on disease-modifying treatments, whereas the IHK model is well suited for studying the antiseizure activity of novel ASDs against difficult-to-treated focal seizures.
1 | INTRODUCTION

There is an ongoing need for developing new antiseizure drugs (ASDs), particularly because about 30% of patients with epilepsy do not adequately respond to current ASDs. For this purpose, animal models of drug-resistant epilepsy are increasingly being used, for instance in the National Institutes of Health (NIH) National Institute of Neurological Disorders and Stroke (NINDS)-sponsored Epilepsy Therapy Screening Program (ETSP) in the USA. Because epilepsy is characterized by spontaneous recurrent seizures (SRSs), chronic epilepsy models with SRSs simulate the disease more closely than models in which single seizures are chemically or electrically induced in normal rodents. Epilepsy with SRSs can be induced in rodents by various means, but models in which SRSs develop after a chemically or electrically induced status epilepticus (SE) are the most widely used models for ASD testing, although the laborious video-electroencephalographic (EEG) monitoring needed for this purpose limits their use. Furthermore, post-SE models of acquired epilepsy are used in the search for drugs that prevent or modify the development of epilepsy by interfering with epileptogenic processes during the latent period following SE.

Among the various available post-SE models of epilepsy, mouse models in which epilepsy is induced by intracerebral injection of the excitotoxic glutamate receptor agonist kainate have become increasingly popular in recent years. Compared to rodent models in which SE is induced by systemic administration of kainate or the muscarinic receptor agonist pilocarpine, epilepsy induced by focal unilateral injection of kainate into either hippocampus or amygdala resembles mesial temporal lobe epilepsy (mTLE) more closely, particularly because the widespread brain damage observed with systemic convulsants is avoided. For unknown reasons, the intra-amygdala kainate (IAK) model has previously been used almost exclusively for studies on epileptogenesis and antiepileptogenesis (AEG), whereas the intrahippocampal kainate (IHK) model has been used for both AEG and ASD studies.

In the present study, we performed a face-to-face comparison of the IAK and IHK mouse models of mTLE, which to our knowledge has not been performed previously. The aim was to compare SE type and duration, mortality, the duration of the latent period after SE, SRS types and frequencies, the sensitivity of SRSs to treatment with the benchmark ASD carbamazepine (CBZ), and hippocampal damage in the two models, using the same mouse inbred strain (C57BL/6). Furthermore, some experiments were performed in the CD-1 outbred mouse strain, mainly because IHK injection into C57BL/6 mice did not result in any clear latent period before onset of SRSs. We found a variety of significant differences between the IAK and IHK models, which is important when deciding which of these models is best suited for studies on antiseizure or antiepileptogenic therapies.

2 | MATERIALS AND METHODS

2.1 | Animals

For the IAK model, male inbred C57BL/6N mice were obtained from Charles River at the age of 7 weeks. For the IHK model, male C57BL/6J mice from either Charles River or Janvier were used. For the latter model, experiments were also performed in male CD-1 mice (Charles River). Furthermore, for the IHK model, previously published data from male NMRI mice (Charles River) were used for comparison with the CD-1 and C57BL/6 mouse strains.

Animals were housed under controlled conditions (ambient temperature = 22-24°C, humidity = 30%-50%, lights on from 6:00 AM to 6:00 PM). Mice were adapted to the laboratory conditions for at least 1 week before being used in experiments. Experiments were performed according to the 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 governmental agency (Lower Saxony State Office for Consumer Protection and Food Safety) responsible for approval of animal experiments in Lower Saxony (reference number for this project: 14/1659). All efforts were made to minimize both the suffering and the number of animals. All animal experiments of this study are reported in accordance with ARRIVE guidelines.8 (For a more detailed description of the mouse strains and principles of animal welfare, see Appendix S1.)

2.2 | The IAK mouse model

Based on various preliminary experiments with different protocols of injecting kainate into the amygdala of conscious or anesthetized mice (see Appendix S1), we decided to inject kainate (Sigma-Aldrich) into the basolateral amygdala (BLA) under anesthesia with chloral hydrate. During anesthesia with chloral hydrate (375 mg/kg intraperitoneal [ip]), kainate monohydrate (0.3 µg in 0.2 µL vehicle) was slowly injected over 60 seconds with a 0.5-µL microsyringe into the right BLA using the following stereotactic coordinates (in mm to bregma): anteroposterior (AP), −1.10; lateral (L), −3.30; dorsoventral (DV), −4.50. After injection, the needle of the syringe was maintained in situ for additional 2 minutes to limit reflux along the injection track. A bipolar EEG recording electrode was placed into the ipsilateral CA1 of the hippocampus using the following coordinates: AP, −2.0; L, −1.50; DV, −1.80. A screw, placed above the left parietal cortex, served as the indifferent reference electrode. Video-EEG monitoring was used to verify the onset, type, and duration of SE, to determine the latent period, and to monitor SRSs following the latent period. (For a more detailed description, see Appendix S1.)

2.3 | The IHK mouse model

For the IHK model, mice were anesthetized with chloral hydrate (375 mg/kg ip in C57BL/6 mice; 500 mg/kg ip in CD-1 mice) and kainate monohydrate (0.21 µg in 50 nL saline) was stereotaxically injected into the right CA1 area of the dorsal hippocampus as described previously.9 (For a more detailed description of the IHK model, see Appendix S1.)

2.4 | Video-EEG monitoring

Continuous (24 h/d) video-EEG monitoring was performed during SE and for at least 1 week after SE as well as for further 1-week periods at different intervals following kainate injection to record the different types of spontaneous electrographic and electroclinical seizures developing after a latent period following IHK or IAK in mice. (For a more detailed description, see Appendix S1.)

2.5 | Types of spontaneous seizures

Three types of SRS were recorded in the two models. In both models, focal and secondarily generalized electroclinical seizures were observed (see Results). Furthermore, as described previously,7,10,11 in the IHK model highly frequent focal electrographic seizures occurred in the EEG recorded from the kainate focus in the CA1. Based on their morphology, these electrographic seizures were differentiated into high-voltage sharp waves (HVSWs) and hippocampal paroxysmal discharges (HPDs) as described by us in detail recently.7 Such electrographic seizures are not observed in sham controls.12 (For a more detailed description, see Appendix S1.)

2.6 | Experiments with CBZ

In the IHK model, the antiseizure effect of CBZ was studied in C57BL/6 and CD-1 mice as previously described for NMRI mice.7 In the IAK model, CBZ had to be administered over several days to allow testing of drug activity on electroclinical seizures, which were much less frequent than the electrographic seizures in the IHK model (see Results). (For a more detailed description, see Appendix S1.)

2.7 | Histology

Following the last video-EEG recording period, the mice were anesthetized and transcardially perfused with paraformaldehyde. (For a detailed description of histological procedures, see Appendix S1.)

2.8 | Statistics

In all experiments, mice were randomly assigned to the drug- and vehicle-treated groups and experiments were performed in a blinded fashion. Depending on whether data were normally distributed, either parametric or nonparametric tests were used for statistical evaluation. (For a more detailed description, see Appendix S1.)

3 | RESULTS

3.1 | Kainate-induced SE in the IHK and IAK mouse models

Following injection of kainate (0.21 µg) into the CA1 of the dorsal hippocampus under anesthesia with chloral hydrate, it took about 3 hours on average before SE started in male
C57BL/6 mice (Table 1). Similar values were obtained in outbred mouse strains without any significant interstrain differences. As reported previously by us and other groups in different mouse strains, the limbic (nonconvulsive) SE induced by kainate was characterized by continuous activity of spikes or spike-and-waves and polyspikes in the ipsilateral hippocampal EEG (Figure S1B). Mice either were immobile or exhibited mild clonic movements of the forelimbs; however, intermittent generalized convulsive seizures (Racine stage 4 or 5) were observed in all mice; these were associated with concomitant high-frequency spikes or polyspikes in the hippocampal recordings. Average SE duration was about 15-18 hours, without significant interstrain differences (Table 1).

Following injection of kainate (0.3 µg) into the BLA under anesthesia with chloral hydrate, it took 2.8 hours on average before SE started in male C57BL/6 mice, which was not different from latency to SE in the IHK model (Table 1). As reported previously by the Henshall group for C57BL/6 mice,13 intra-amygdala injection of 0.3 µg kainate-induced limbic seizures, with secondary generalization characterized by head bobbing, facial and forelimb clonus, and prolonged, generalized tonic-clonic activity, with loss of postural control. Overall, the SE induced by IAK was more severe than the SE induced by IHK, which, at least in part, may be a consequence of the difference in kainate dose (0.3 vs 0.21 µg). On the EEG, the SE induced by IAK was characterized by continuous activity of spikes or spike-and-waves and polyspikes in the ipsilateral hippocampus (Figure S2B). The polyspikes were interrupted by frequent generalized convulsive seizures as shown in Figure S2B. Average SE duration was 12.5 hours, which was not significantly different from the data obtained with the IHK model (Table 1).

With respect to mortality during and after SE, the IAK and IHK models differed significantly, which could be because of the more severe SE in the IAK model and because we did not suppress SE by benzodiazepines (Table 1). Thus, in C57BL/6 mice mortality was 44% in the IAK model versus 0% in the IHK model (P = .016). Low mortality was also observed when using outbred mice for the IHK model (Table 1).

### 3.2 Latent period following kainate-induced SE in the IHK and IAK mouse models

Following SE, the duration of the latent period to onset of SRSs in the IHK model depended on whether electrographic or electroclinical seizures were used for defining onset of SRSs (Table 2). In this model, most groups do not analyze the infrequent electroclinical seizures, but only the more frequent focal electrographic seizures recorded from the kainate focus in the ipsilateral hippocampus. When doing so, no
| Model | Mouse strain | Kainate dose, µg | Clear latent period after SE? | First HVSWs, days after SE, mean (range) | First HPDs, days after SE, mean (range) | First electroclinical seizures, days after SE, mean (range) | Frequency of HVSWs, HPDs, and electroclinical seizures in chronic epileptic phase | Percentage of mice with Electrographic seizures, % | Electroclinical seizures, % |
|-------|--------------|-----------------|-------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------------|-----------------------------------------------|---------------------------------|--------------------------|
| IAK   | C57BL/6      | 0.3             | Yes 3.4 (1-7) days            | None                                     | None                                     | 4.4 (2-8)                                        | None                                           | None                             | 0                        |
| IAK   | C57BL/6      | 0.21            | No 2.1 (0-7) days            | 3.1 (1-8)                                | 3.1 (1-10)                               | 3.6 (1-9)                                        | 17.5 ± 7.3                                      | 3.5 ± 3.9                      | 1.1 ± 0.7                |
| IAK   | CD-1         | 0.21            | Yes 9.9 (8-13) days*         | 10.9 (9-14)***                          | 12.0 (11-14)***                         | 3.8 (1-7)                                        | 7.1 ± 9.1**                                     | 8.9 ± 12.5                      | 1.1 ± 1.7                |
| IAHK  | NMRI         | 0.21            | Yes 4.8 (4-6) days*          | 5.8 (5-7)*                               | 12.0 (10-14)***                         | 7.5 (3-12)                                       | 28.8 ± 6.1                                      | 11.6 ± 4.0                      | 0.9 ± 0.9                |

Note: In both models, kainate was injected under anesthesia with chloral hydrate and SE was not terminated. Data for the IHK model in NMRI mice were taken from a previous study9 and are included here for comparison.

Abbreviations: EEG, electroencephalography; HPD, hippocampal paroxysmal discharge; HVSW, high-voltage sharp wave; IAK, intra-amygdala kainate; IHK, intrahippocampal kainate; SE, status epilepticus.

Significant differences to C57BL/6 mice in IHK model:

*P < .05.

**P < .01.

***P < .001.

****P < .0001.

Significant difference between IAK and IHK model:

****P < .0001.
obvious latent period was determined in several (5/14) of the C57BL/6 mice (range = 0-7 days). In contrast, a relatively long, seizure-free latent period of 8-13 days was obtained in CD-1 mice and 4-6 days in NMRI mice, resulting in significant differences in latent period between mouse strains (Table 2). As previously described, the first electrographic seizures recorded from the kainate focus in the ipsilateral hippocampus were HVSWs, followed later by HPDs (Table 2). However, in some mice electroclinical seizures were observed before the electrographic seizures, resulting in a shorter latent period. It is not clear whether these early electroclinical seizures were acute, insult-associated seizures or first SRSs. The stepwise progression of SRSs from electrographic to electroclinical (convulsive) seizures during the latent period shown by Heinrich et al. would suggest that electroclinical seizures occurring before electrographic seizures are acute insult-associated seizures and not first SRSs.

In the IAK model, such distinction between electrographic and electroclinical seizures was not possible, because, in contrast to the IHK model, no electrographic seizures were recorded from the CA1 in the ipsilateral hippocampus (Figure S2). Thus, the duration of the latent period had to be based on first occurrence of electroclinical seizures, which ranged between 2 and 8 days following SE, resulting in a latent period of 1-7 days, which was not significantly different from the short or absent latent period in the IHK model in C57BL/6 mice (Table 2).

### 3.3 Development of epilepsy following kainate-induced SE in the IHK and IAK mouse models

Following the latent period, spontaneous electrographic and electroclinical seizures were observed in the IHK model (Figure S1; Table 2), whereas only electroclinical seizures were determined in the IAK model (Figure S2; Table 2). As reported previously, the electrographic seizures in the IHK model consisted of highly frequent HVSWs and HPDs (Figure S1F,G), whereas electroclinical seizures occurred only infrequently (Table 2). Most electroclinical seizures were secondarily generalized convulsive (tonic-clonic) seizures, but focal seizures were also observed (Figure S1C,D). In the IAK model, electroclinical seizures were recorded more frequently than in the IHK model (Table 2). As in the IHK model, most electroclinical seizures in the IAK model were secondarily generalized convulsive seizures, but focal seizures were also observed (Figure S2C,D). The average frequency of electroclinical seizures was significantly higher in the IAK model (~4 seizures/d) than in the IHK model (~1 seizure/d; Table 2).

Figure S3 shows a heat map of six epileptic mice of the IAK model in which SRSs were video-EEG monitored (24/7) over 1 week at both 4 and 8 weeks after SE. The heat map illustrates two important features of the model. First, seizure frequency was highly variable, with daily seizure rates ranging between 33 and 1. Second, SRS frequency did not progress during the 2 months of observation but rather declined. Average SRS frequency of the 6 mice at 4 weeks after SE was 6.9 (range = 1.7-14) seizures/d versus 1.9 (0.4-4.4) seizures/d at 8 weeks after SE ($P = .0811$). This was different in the IAK model, in which SRS frequency did either not change or tended to increase as a function of time after SE.

In addition to SRSs, interictal spikes were observed in the hippocampal EEG in both models without obvious differences between the IHK and IAK model (Figures S1E and S2E).

### 3.4 Neuronal damage in the hippocampus in the IHK and IAK mouse models

In the IHK model, neuropathology was restricted to the ipsilateral hippocampus and was characterized by neuronal loss in CA1, CA3, and dentate hilus (Figure 1C). In addition, marked granule cell dispersion was observed, which is characterized by progressive enlargement of the dentate gyrus with a striking radial dispersion of the granule cells associated with reactive astrocytes. The amygdala is not damaged in this model (Figure 1F). Furthermore, the contralateral hippocampus does not exhibit any obvious abnormal structural alterations, which was also observed in the IAK model (Figure 1A). In the IAK model, cell loss was observed in the ipsilateral BLA (Figure 1E), associated with marked neuronal loss in the ipsilateral hippocampus, mainly in ipsilateral CA3 and hilus (Figure 1B). The contralateral BLA (Figure 1D) appeared normal.

### 3.5 Effect of CBZ on spontaneous seizures in the IHK and IAK mouse models

Because mTLE is characterized by focal seizures, which are often resistant to ASDs, the highly frequent focal electrographic seizures in the IHK model are commonly used to test the efficacy of ASDs. We have previously reported that CBZ, at 20 and 40 mg/kg ip, significantly reduced the frequency of different types of focal (hippocampal) electrographic seizures in this model when using NMRI mice. For comparison with C57BL/6 and CD-1 mice, these data on NMRI mice are included in Figure 2. In C57BL/6 mice, no significant drug effects were observed at 20 mg/kg (Figure 2A), whereas 40 mg/kg reduced the frequency of HVSWs significantly (Figure 2B). The frequency of HPDs was too low...
or irregular in C57BL/6 mice to allow determining CBZ’s effects on this type of electrographic seizure. Similar effects of CBZ were observed in CD-1 mice (Figure 2C,D). In NMRI mice, CBZ reduced the frequency of HPDs but not HVSWs at 20 mg/kg, whereas both types of focal seizures were significantly suppressed at 40 mg/kg (Figure 2E,F). However, the latter dose is above the neurotoxic TD50 (33 mg/kg ip), which has been determined in NMRI mice previously. In accordance with this TD50, at 20 mg/kg, CBZ was tolerated without any obvious behavioral adverse effects, whereas sedation and ataxia were observed at 40 mg/kg.

In addition to analyzing drug effects on seizure frequency, cumulative duration of electrographic seizures can be used as a measure of drug efficacy. It has been shown that some drugs may reduce the frequency of electrographic seizures but increase their duration. For CBZ, effects on cumulative seizure duration (Figure S4) were similar to effects on seizure frequency (Figure 2).

In the IAK model, as described above, electrographic seizures were not observed on the EEG recorded from the CA1 in mice. Instead, the frequency of electroclinical seizures was much higher in the IAK than IHK model, so drug effects on electroclinical seizures were determined during prolonged treatment with CBZ (Figure 3). During a 5-day baseline monitoring period, the frequency of electroclinical seizures ranged from 0.4 to 6.2 seizures/d in eight individual mice. Treatment with CBZ significantly reduced seizure frequency on day 1 and 4 of a 5-day treatment period (Figure 3A). On these days, all eight mice were seizure-free, whereas six of these mice had exhibited seizures on days 1 and 4 of the baseline period (P = .00189). No significant treatment effect was obtained on days 2 and 3, which was mainly due to one mouse (mTAR284) that did not respond to CBZ on these days; this mouse was the animal with the highest seizure frequency during the baseline period. On day 5, five of eight of the CBZ-treated mice exhibited seizures, which was significantly different from the zero of eight mice with seizures on day 1 (P = .0092), whereas the other treatment days did not significantly differ from day 1. The loss of efficacy at day 5 may thus indicate that tolerance to the antiseizure activity of CBZ developed toward the end of the treatment period, which has been observed with CBZ in various other seizure or epilepsy models and is due to both functional and metabolic tolerance developing during prolonged treatment with this ASD.

When data of all 5 days were averaged, CBZ exhibited a significant effect on seizure frequency (Figure 3B). However, relatively high doses of CBZ were administered three times daily (30, 30, and 45 mg/kg, respectively) to account for the rapid elimination of CBZ in mice (half-life is ~3 hours in male animals of this species). Despite these doses, we did not observe any behavioral adverse effects during treatment. When mice were treated with the CBZ vehicle instead of CBZ, no effect on seizure frequency was observed (Figure 3C,D).
In the present study, we performed a face-to-face comparison of the IAK and IHK mouse models of mTLE with the aim of characterizing their utility for pharmacological studies. Table 3 summarizes the most important similarities and differences of the two models when injecting kainate into male C57BL/6 mice. Furthermore, in addition to C57BL/6 mice, the IHK model was characterized in a widely used outbred mouse strain (CD-1) to determine the impact of mouse genetics in this model, particularly with respect to the latent period. Previously reported data in another popular outbred strain (NMRI) were included for comparison.

**FIGURE 2** Effect of carbamazepine (CBZ) on electrographic seizures in epileptic C57BL/6 mice (A,B), CD-1 (C,D), and NMRI mice (E,F) of the intrahippocampal kainate (IHK) model. Epileptic activity was recorded from an electrode located at the kainate injection site in the CA1 region of the dorsal hippocampus. The effect of CBZ was separately assessed for high-voltage spike waves (HVSWs) and hippocampal paroxysmal discharges (HPDs). For each mouse strain, the effect of two CBZ doses (20 mg/kg and 40 mg/kg intraperitoneal) is shown. Data are shown as mean ± SEM per group and were recorded in the 2 hours before and after a single dose of CBZ. Group size was seven (C57BL/6), 11 (CD-1) or six (NMRI), but some mice had to be excluded from final analysis because they did not exhibit HVSWs or HPDs during the predrug control period. Statistical differences between predrug control and postdrug values within each group are indicated by asterisks (*P < .05). Data for the IHK model in NMRI mice were taken from a previous study and are included here for comparison. EEG, electroencephalographic.
Different types of spontaneous seizures and their pharmacology in the IAK and IHK models

The IAK model of mTLE was first described and characterized in the 1970s in rats and only some 25 years later in mice. Since the mouse IAK model was described by David Henshall's group in 2002, this model has become widely used to study the molecular mechanisms of neuronal cell death, epileptogenesis, and pharmacological strategies to prevent epilepsy after brain injury. However, in contrast to the IHK mouse model of mTLE, the pharmacological profile of ASD response in epileptic mice of the IAK model is unknown.

Because of the highly frequent spontaneous focal (electrographic) seizures in the IHK model of mTLE and their resistance to several ASDs, this model has been proposed to present a valuable tool in the search for more effective ASDs, which led to its inclusion in the NIH/NINDS-sponsored ETSP in the USA. This is supported by the present data on CBZ in three mouse strains, in which electrographic seizures, particularly HVSWs, were only suppressed at doses in the neurotoxic range of this benchmark ASD. In contrast, the pharmacology of the relatively frequent spontaneous electroclinical seizures in the IAK model of mTLE is unknown. As shown in this study, these seizures can be suppressed by prolonged treatment with CBZ, but relatively high doses are needed because of the rapid elimination of this ASD in mice. The data for CBZ indicate that...
the spontaneous electroclinical seizures in the AHK model are resistant to tolerable doses of a benchmark ASD, but dose-response experiments and data for other clinically used ASDs are needed for further characterization of the drug response of SRSs in this model. In a previous study by Iori et al22 in the IAK model in C57BL/6 mice, CBZ was administered via the food at doses of 10, 20, and 40 mg/daily/mouse, which corresponds to daily doses of about 300, 600, and 1200 mg/kg body weight, that is, far above the daily dose of 105 mg/kg used in the present study. Despite these very high daily doses, plasma CBZ levels were within the therapeutic range (4-10 µg/mL) when using daily doses of 20 and 40 mg CBZ per mouse,22 indicating low bioavailability following administration of high oral doses of CBZ. Subsequently, a daily dose of 20 mg/mouse/d (ie, ~600 mg/kg body weight) was administered in mice over 2 weeks after onset of SRSs, but the potential effect on SRSs during treatment was not statistically analyzed, because the goal of the study of Iori et al22 was to determine whether treatment with CBZ after onset of epilepsy has an effect on the later progression of epilepsy in this model, that is, a disease-modifying effect.

More recently, West et al23 reported in an abstract that the electroclinical seizures in the IAK model are resistant to CBZ (30 mg/kg three time daily) and phenytoin (20 mg/kg twice daily), substantiating the conclusion of the present study that electroclinical seizures in this model are resistant to standard ASDs. This is an interesting difference from the IHK model, in which we found previously that the infrequent electroclinical seizures respond to ASDs, whereas the much more frequent focal electrographic seizures are resistant to several ASDs, including CBZ and phenytoin,24 thus reflecting the ASD resistance of such focal seizures in patients with mTLE.

### Table 3

| IAK model | IHK model |
|-----------|-----------|
| **Sex**   | Male      | Male     |
| **Age, wk** | 8         | 8        |
| **Kainate injected under anesthesia with** | Chloral hydrate | Chloral hydrate |
| **Kainate injected in** | Basolateral amygdala | CA1 |
| **Dose of kainate** | 0.3 µg (=1.4 nmol/L; in 0.2 µL) | 0.21 µg (=0.92 nmol/L; in 0.05 µL) |
| **EEG recorded from** | CA1 | CA1 (focus) |
| **Onset of SE after** | ~3 h | ~3 h |
| **Type of SE induced** | Convulsive SE | Mostly nonconvulsive SE |
| **Termination of SE by benzodiazepines** | No | No |
| **Duration of SE** | ~12 h | ~15 h |
| **Mortality** | 44% | 0% |
| **Duration of latent period** | ~3 (1-7) d | ~2 (0-7) d |
| **Types of spontaneous seizures** | Electroclinical (focal and secondarily generalized) | Electrographic and electroclinical (focal and secondarily generalized) |
| **Frequency of electrographic seizures per hour** | No electrographic seizures recorded from CA1 | Highly frequent |
| **Frequency of electroclinical seizures** | >4 per day | ~1 per day |
| **Progression of epilepsy** | Not observed | Not observed |
| **Relevant differences for antiseizure drug testing** | Readout is electroclinical seizures; occur relatively frequently, but longer video-EEG monitoring and longer drug treatment to assess drug efficacy compared to IHK model | Readout is electrographic seizures; occur highly frequently, which enables acute drug testing (without prolonged video-EEG monitoring and drug treatment) |
| **Sensitivity of seizures to carbamazepine** | Yes (but only at high doses) | Yes (but only at high doses) |

**Note:** For details, see Tables 1 and 2 and text.

Abbreviations: EEG, electroencephalogram; IAK, intra-amygdala kainate; IHK, intrahippocampal kainate; SE, status epilepticus.
In contrast to the highly frequent electrographic seizures recorded from the kainate focus in the ipsilateral CA1 in the IHK model, such seizures were not observed in the IAK model. However, Detlev Boison’s group reported that frequent electrographic seizures can be recorded in the IAK mouse model from the ipsilateral BLA and hippocampal CA3 with an average frequency of 4/h, whereas such seizures cannot be recorded from CA1 or dentate gyrus. The high frequency of electrographic seizures recorded from BLA and CA3 may allow acute drug testing similar to that performed in the IHK model, which is an interesting aspect for further studies.

4.2 Technical factors that may cause interlaboratory differences in kainate models

In previous studies in the IAK model, kainate was injected into awake mice via a previously implanted guide cannula and the SE was interrupted after 30 or 40 minutes by a benzodiazepine to reduce mortality and restrict hippocampal damage. Due to problems while establishing this protocol in our laboratory (see Appendix S1), we decided to inject kainate into anesthetized mice, which simplifies the protocol, because no previous surgery with guide cannula implantation is needed. Furthermore, we skipped the SE suppression by a benzodiazepine, because this would have affected the face-to-face comparison with the IHK model, in which SE is generally not interrupted by an ASD. The most likely explanation for our initial difficulties in establishing the IAK model are laboratory-to-laboratory differences in the technique of local kainate infusion and between C57BL/6 substrains used by different groups. In the studies of David Henshall’s group, male C57BL/6j(OlaHsd) mice from Harlan UK or C57BL/6 mice from Charles River (substrain not defined) were used, whereas Annamaria Vezzani’s group used male C57BL/6N mice from Charles River in Italy and we used C57BL/6N mice from Charles River in Germany. We and others have shown previously that different C57BL/6 substrains or the same substrain from different vendors or even from different barriers of the same vendor may markedly differ in their susceptibility to convulsants. Furthermore, although the C57BL/6j(OlaHsd) and C57BL/6N substrains originally descended from the same C57BL/6 breeding stock and were maintained at the Jackson Laboratory in Bar Harbor, Maine, their subsequent history differed, leading to distinct substrains.

Another factor that may cause interlaboratory differences in kainate models is the source and storage of kainate. Kainic acid ((2S,3S,4S)-3-(carboxymethyl)-4-(prop-1-en-2-yl)pyrrolidine-2-carboxylic acid), or kainate, is a conformationally restricted cyclic analog of L-glutamic acid and the prototype agonist at the kainate class of ionotropic glutamate receptors. It was originally isolated from a red marine alga (Digenea simplex) found in tropical and subtropical waters, but worldwide shortages of this seaweed natural product in the year 2000 prompted numerous enantioselective syntheses. The synthesis of kainate represents a considerable challenge because of the three contiguous chiral centers of the pyrrolidine ring. The biological activity of kainate is linked to the trans-C2/C3/cis-C3/C4 stereochemistry, and thus, any synthesis should result in efficient control of this relative stereochemistry. Currently, both synthetic and seaweed-derived versions of kainate are commercially available. As a consequence, the neurotoxic activity of kainate may vary as a function of source and batch. Furthermore, because kainate is quite expensive, most laboratories do not prepare solutions freshly on each experimental day but rather use stock solutions that are stored frozen. Details are rarely given, although kainate is known to be sensitive to light and temperature and may degrade upon repeated thawing and freezing.

4.3 Differences in basic features of the IAK and IHK models

In the present study, significant differences between the basic features of the IAK and IHK models were found (Table 3). As a possible consequence of most studies, including the present study, using higher doses of kainate in the IAK than in the IHK model, the SE in the IAK model is more severe, resulting in a high mortality rate, particularly when SE duration is not reduced by an ASD. The reason for this difference in intracerebrally infused dose of kainate is that ~40% higher doses of kainate are needed to induce hippocampal damage following infusion into the BLA compared to kainate infusion into the hippocampus. The more severe SE in the IAK model is the reason most groups suppress SE by benzodiazepines in this model, whereas this is typically not done in the IHK model. The hippocampal damage in the IAK and IHK models differed in that neuronal loss in the ipsilateral hippocampus in the IAK model was less widespread than in the IHK model. In the IAK model, neuronal loss was mainly observed in the BLA, hippocampal CA3/CA4 region, and dentate hilus, whereas CA1, CA3, and hilus were severely affected in the IHK model, most likely as a consequence of direct infusion of the excitotoxic kainate into the CA1. Furthermore, granule cell dispersion occurred in the IHK but not in the IAK model. Overall, these findings in the IAK and IHK models were in line with previous reports.

The latent period to onset of SRSs was very short, if not absent, in several animals of the IAK and IHK models when using male C57BL/6 mice, whereas a longer latent period was observed in the IHK model in male NMRI and CD-1 mice. For the IHK model, a lack of any clear latent period...
4.4 | Mouse strain differences

Interestingly, except for the latent period, the mouse strain (C57BL/6, CD-1, NMRI) hardly affected the characteristics of the IHK model, whereas this had not yet been studied for the IAK model. For the latter model, most previous studies used C57BL/6 mice from different vendors, but some experiments were also performed in Balb/c mice and SJL mice. Interestingly, whereas the C57BL/6 strain is often considered more resistant to induction of seizures than other mouse strains, this does not seem to apply to intracerebral injection of kainate. In contrast to other mouse strains, systemically administered kainate fails to induce hippocampal damage in C57BL/6 mice, which can be overcome by intra-amygdala or intrahippocampal injection of kainate. On the other hand, systemic administration of pilocarpine exerted very similar consequences (SE, SRSs, hippocampal damage) in C57BL/6 mice and outbred CD-1 mice.

4.5 | Consequences for pharmacological studies in the IAK and IHK models

Figure 4 illustrates how the IHK and IAK models fulfill crucial requirements of useful animal models of mTLE for studying the activity either of ASDs or of antiepileptogenic treatments. If one assumes that structural and functional changes in the hippocampus are mainly responsible for epileptogenesis in both models, then the IAK model may...
provide an advantage for pharmacological studies on antiepileptogenesis or disease modification, as the hippocampus is not immediately damaged by the intra-amygdala injection of kainate, but instead hippocampal damage is a consequence of prolonged SE. In contrast, IHK injection directly and almost immediately causes hippocampal damage by the neurotoxic effect of the glutamate receptor agonist, although SE contributes to the overall damage. Thus, onset of pharmacological treatment several hours after IHK injection, as typically done in studies evaluating drugs for antiepileptogenic efficacy, may be too late to prevent hippocampal damage and epileptogenesis. This problem is illustrated by our recent experiments with N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists, which did not prevent neuronal damage and epileptogenesis in the IHK mouse model when treatment was initiated 6-8 hours after kainate, whereas NMDA and AMPA receptor antagonists prevent kainate-induced excitotoxic cell death when administered before or shortly after the convulsant.

With respect to testing ASDs, the present study indicates that the IAK model is an interesting approach to evaluating the response of electroclinical seizures to ASDs, but such experiments are laborious, because prolonged continuous (24/7) video-EEG recording is needed, which is a disadvantage compared to models with more frequent focal SRSs, such as the IHK model (Figure 4), or models with induced seizures, such as the 6-Hz mouse model of partial seizures. Thus, the main use of the IAK model may be in the evaluation of novel antiepileptogenic or disease-modifying treatments. Here, it is also important that—the same as the IHK model—behavioral and cognitive alterations develop in the IAK model, which can be used as additional readouts in studies on disease modification, although the cognitive deficits in the IAK model appear to be less pronounced than in the IHK model.

However, for studies on antiepileptogenic or disease-modifying treatments it should be considered that reduction of SE severity by a benzodiazepine, as done by most groups using the IAK model, may interfere with the efficacy of the novel antiepileptogenic or disease-modifying treatment, as previously shown for the pilocarpine model of mTLE. A further problem is that focal kainate models lack etiological relevance to human mTLE, which, however, is not necessarily important if the predictions of the models can be validated by etiologically and clinically more relevant preclinical models, such as the emerging mouse and rat models of traumatic brain injury.

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CONFLICT OF INTEREST
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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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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