

The effects of carbamazepine in the intrahippocampal kainate model of temporal lobe epilepsy depend on seizure definition and mouse strain

*†Friederike Twele, *†Kathrin Töllner, *†Marion Bankstahl, and *†Wolfgang Löscher

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SUMMARY

Objective: Mesial temporal lobe epilepsy (TLE) with hippocampal sclerosis is a predominant form of acquired epilepsy, characterized by recurrent simple and complex partial seizures that are often resistant to treatment. Mice developing spontaneous recurrent nonconvulsive and convulsive seizures after intrahippocampal injection of the excitotoxic glutamate agonist kainate are thought to represent a valuable model of mesial TLE. Epileptic electroencephalogram (EEG) activity recorded in this model from the kainate focus in the ipsilateral hippocampus is resistant to antiseizure drugs such as carbamazepine (CBZ). We compared the efficacy of CBZ in this model in two different mouse strains (FVB/N and NMRI). Furthermore, we evaluated whether changes in the definition of electrographic seizures affect the antiseizure efficacy of CBZ.

Methods: As in previous studies, two types of epileptic EEG activity were defined: high-voltage sharp waves (HVSWs) and hippocampal paroxysmal discharges (HPDs). The characteristics of these paroxysmal EEG events in epileptic mice were compared with EEG criteria for nonconvulsive seizures in patients. For HVSWs, different spike frequencies, interevent intervals, and amplitudes were used as inclusion and exclusion criteria. In addition to CBZ, some experiments were performed with diazepam (DZP) and phenobarbital (PB).

Results: Female epileptic FVB/N mice predominantly exhibited frequent HVSWs, but only infrequent HPDs or secondarily generalized convulsive seizures. Slight changes in HVSW definition determined whether they were resistant or responsive to CBZ. Male NMRI mice exhibited both HVSWs and HPDs. HVSWs were more resistant than HPDs to suppression by CBZ. Both types of epileptic EEG activity were rapidly suppressed by DZP and PB.

Significance: The data demonstrate that focal electrographic seizures in the intrahippocampal kainate mouse model are less resistant than previously thought. Both mouse strain and the criteria chosen for definition of EEG seizures determine whether such seizures are drug-resistant or -responsive.

KEY WORDS: Antiseizure drugs, Pharmacoresistance, Electrographic seizures, Diazepam.



Friederike Twele is a postdoctoral researcher in Dr. Löscher's lab.

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*Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Hannover, Germany; and †Center for Systems Neuroscience, Hannover, Germany

Address correspondence to Wolfgang Löscher, Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany. E-mail: wolfgang.loescher@tiho-hannover.de

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KEY POINTS

- The intrahippocampal kainate mouse model of temporal lobe epilepsy is characterized by highly frequent spontaneous electrographic seizures
- This allows assessing antiseizure drug effects on spontaneous seizures by only 1–2 h of EEG recording following drug injection
- We examined how drug effects on epileptic hippocampal EEG activity are affected by seizure definition and mouse strain
- Both variables critically determined whether electrographic seizures were responsive or resistant to carbamazepine
- Thus, similarly as for patients, the genetic background of mice has a critical influence on the drug response of seizures in this model

The intrahippocampal kainate mouse model of mesial temporal lobe epilepsy (TLE) is increasingly used in the search for antiseizure drugs (ASDs) with potential activity against therapy-resistant epilepsy as well as for drugs that may prevent or modify development of epilepsy after brain injury.^{1–3} Induction of status epilepticus (SE) by unilateral intrahippocampal injection of kainate is associated with almost no mortality, and most mice develop ipsilateral hippocampal damage and highly frequent nonconvulsive or electrographic and less-frequent convulsive seizures after SE.^{2,4,5} Riban et al.⁵ were the first to report that epileptic electroencephalogram (EEG) activity recorded from the ipsilateral hippocampus in this model is resistant to major ASDs such as carbamazepine (CBZ), phenytoin (PHT), and valproate (VPA), whereas diazepam (DZP) was capable of suppressing these focal electrographic events. Using male Swiss mice, Riban et al.⁵ described two types of chronic epileptic activity in the EEG recorded from the ipsilateral hippocampus: (1) high-voltage sharp waves (HVSWs), which were grouped in bursts (3–6 Hz, 1.5–4.5 mV, 4–10 s); and (2) hippocampal paroxysmal discharges (HPDs), which were characterized by higher frequency (10–14 Hz), lower amplitude (0.7–1.1 mV), and longer duration (20–60 s) than the HVSWs (Table 1). The finding that these paroxysmal discharges recorded from the ipsilateral hippocampus are resistant to ASDs such as CBZ and PHT was confirmed by subsequent studies,^{6,7} so that Guillemain et al.¹ proposed that the intrahippocampal mouse model of mesial TLE is suited as a model of difficult-to-treat focal seizures. In this model, because of the high recurrence of paroxysmal hippocampal discharges, ASDs can easily be tested during the 1–2 h that follow ASD injection and also during chronic treatment.^{1,3} This is a major advantage over other animal models of epilepsy with spontaneous seizures, in which, because of the low frequency of the seizures,

continuous (24/7) video/EEG recordings over weeks are needed for drug efficacy studies.³ Based on the interesting features of the intrahippocampal kainate mouse model, it is currently being evaluated as a model of therapy-resistant mesial TLE by the Anticonvulsant Screening Program (ASP) of the University of Utah, which is sponsored by the National Institutes of Health (NIH) National Institute of Neurological Disorders and Stroke (NINDS).

On the basis of data from the 6-Hz mouse model of intractable partial seizures, which is another model used by the ASP for drug screening, Leclercq and Kaminski⁸ recently demonstrated that the genetic background of mice and experimental conditions strongly influence treatment resistance so that the initial observation about treatment resistance of 6-Hz seizures⁹ should be interpreted with strain and experimental conditions in mind. The aim of the present study was therefore to evaluate whether the mouse strain also affects the pharmacology of epileptic EEG activity in the intrahippocampal kainate model. We chose CBZ for this study because it was repeatedly reported to be ineffective against epileptic EEG activity in this model.^{5,6,10} In contrast to these previous studies, the effect of CBZ was separately evaluated for HVSWs and HPDs to examine whether these paroxysmal EEG events respond differently to treatment. Furthermore, we examined whether changes in the arbitrary definition of EEG seizures have an impact on the outcome of pharmacological treatment.

MATERIALS AND METHODS

Animals

Outbred male NMRI (Naval Medical Research Institute) mice, which originated from a colony of Swiss mice and which are used as a general-purpose stock in many fields of research including pharmacology,¹¹ were obtained from Charles River (Sulzfeld, Germany) and inbred female FVB/N mice from Taconic (Ejby, Denmark) at an age of 4–7 weeks (body weight 20–22 g). FVB/N mice also originated from an outbred colony of Swiss mice (N:GP; NIH general-purpose mouse) established at the NIH in 1935.¹² Owing to the prominent pronuclei in their fertilized eggs and the large litter size, FVB/N mice are commonly used for transgenic experiments and subsequent genetic analyses.¹² We used FVB/N mice previously for ASD testing in the intrahippocampal kainate model.¹⁰ Mice were adapted to the laboratory conditions for 1–2 weeks before being used in experiments, so all mice were midadolescent at the 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. Female animals were housed without males to keep them acyclic or asynchronous with respect to their estrous cycle.^{13,14}

Table 1. Comparison of the characteristics of the two main types of spontaneous hippocampal EEG events, high-voltage sharp waves (HVSWS) and hippocampal paroxysmal discharges (HPDs), reported by different groups in different strains of mice in the intrahippocampal kainate model of mesial temporal lobe epilepsy

Type of epileptic EEG activity	Riban et al. ⁵ (male Swiss mice)	Maroso et al. ⁷ (male C57BL/6 mice)	Duveau et al. ³⁵ (male C57BL/6 mice)	Töpfer et al. ⁵⁵ (female N1MRI mice)	Klein et al. ¹⁰ (female FVB/N mice)	Twele et al. ¹⁹ (female N1MRI mice)	Twele et al. ¹⁹ (male N1MRI mice)	Present study (female FVB/N mice) ^a	Present study (male N1MRI mice) ^a
HVSW									
Morphology	Sharp waves (monomorphic)	Sharp waves (monomorphic)	Not reported	Sharp waves (monomorphic)	Sharp waves (monomorphic)	Sharp waves (monomorphic)	Sharp waves (monomorphic)	Sharp waves (often monomorphic)	Sharp waves (often monomorphic)
Intraevent evolution	No	No	—	Not reported	Not reported	Not reported	Not reported	Mostly no	Mostly no
Spike frequency	3–6 Hz	3–8 Hz	—	≥1 Hz	≥1 Hz	≥1 Hz	≥1 Hz	≥2 Hz	≥2 Hz
Amplitude	1.5–4.5 mV	1–4 mV	—	≥2 times baseline	≥2 times baseline	≥2 times baseline	≥2 times baseline	≥3 times baseline	≥3 times baseline
Duration	4–10 s	~20 s ^c	—	≥3 s	≥3 s	≥3 s	≥3 s	≥5 s	≥5 s
Interevent interval	Not reported	Not reported	—	≥2 s	Return to baseline	≥2 s	≥2 s	≥3 s	≥3 s
Frequency of HVSWS	Not reported	Not reported	—	40–100/h	20–100/h	30–60/h	10–40/h	1–40/h	2–28/h
HPD									
Morphology	Spikes and polyspikes (polymorphic)	Spikes and polyspikes (polymorphic)	Rhythmic high-amplitude sharp waves	Only rarely observed	Only rarely observed	Spikes and polyspikes (polymorphic)	Spikes and polyspikes (polymorphic)	Only rarely observed	Spikes and polyspikes (polymorphic) ^a
Intraevent evolution	Yes	Yes	No reported	—	—	Yes	Yes	—	Yes
Spike frequency	10–14 Hz ^d	10–20 Hz ^d	Not reported	—	—	Not determined (definition only based on morphology)	Not determined (definition only based on morphology)	—	≥5 s with ≥5 Hz, <3 s interruption with <5 Hz
Amplitude	0.7–1.1 mV	0.5–1 mV	>2 times baseline	—	—	≥2 times baseline	≥2 times baseline	—	Based on evolution ≥2–3 times baseline ^e
Duration	20–60 s	30–60 s ^c	≥5 s	—	—	~45 s	~45 s	—	5–>20 s ^e
Interevent interval	Not reported	Not reported	1 s	—	—	≥2 s	≥2 s	—	≥3 s
HPD frequency	Max. 60/h ^b	Not reported	38 ± 3/h	—	—	0.2–5.0/h	5–26/h	—	0–17/h

^aFor details see text and Table 2.

^b30–100/h reported in a subsequent study by the same group.⁵⁶

^cSpike trains with a frequency of 1–3 Hz and/or duration <20 s were not considered in the quantitative analysis of epileptic activity.

^dThis high frequency relates to only the low-amplitude, high-frequency part of the HPDs, but not to the slower high-amplitude spike-and-wave pattern with which most HPDs start.

^e5–20 s = short HPD; >20 s = long HPD.

Experiments were performed according to the EU council directive 210/63/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, SE is induced by unilateral injection of kainate into the CA1 sector of the dorsal hippocampus.^{15,16} For this purpose, mice were anesthetized with chloral hydrate (400–500 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 the right CA1 area of the dorsal hippocampus as described previously.¹⁷ Kainate was slowly injected over 60 s with a 0.5-µl microsyringe. In preliminary experiments in groups of six mice, stereotaxic coordinates (according to Paxinos and Franklin¹⁸) were determined for the two mouse strains (FVB/N and NMRI) by histological verification of injection site in CA1 (see Twele et al.¹⁹). These coordinates were then used for the experiments described in this study. 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 Gröticke et al.¹⁷). During all surgical procedures and for about 1 h thereafter, mice were kept on a warming pad to avoid hypothermia.

Video/EEG recording

After surgery, EEG/video monitoring started to verify the limbic, predominantly nonconvulsive SE induced by kainate. Starting about 8 weeks after SE, video-EEG monitoring (24/7) was used to verify that all mice had developed epilepsy.

For EEG recording, mice were connected via a flexible cable to a system consisting of 8 one-channel bioamplifiers (ADInstruments Ltd., Sydney, NSW, Australia) and an analog-digital converter (PowerLab 8/30 ML870; ADInstruments). The data were recorded and analyzed with LabChart 6 for Windows software (ADInstruments), at a sampling rate of 200 Hz, a time constant of 0.1 s, low-pass filter of 60 Hz, and a 50-Hz notch filter. The EEG recording was directly linked to simultaneous digital videorecording using one high-resolution infrared camera for up to eight mice (NYCTO Vision; CaS Business Services, Wunstorf, Germany). For video/EEG monitoring, mice were housed singly in clear acrylic cages (one per cage). For monitoring

during the dark phase, infrared LEDs were mounted above the cages.

All EEGs were visually examined for abnormal electrographic activity. HVSWs, HPDs, and interictal EEG activity were defined as described in the Results section. If not otherwise defined, the term “frequency [Hz]” as a parameter for criteria defined by our group is the “mean frequency of discharges in an EEG event,” that is, number of spikes divided by duration of the event. The infrequently occurring secondarily generalized convulsive seizures were not quantitated, but they occurred in most mice, thus clearly indicating that the mice were epileptic.

Drug testing

Starting 4–6 months after SE, that is, in the chronic phase of epilepsy in this model, the effect of CBZ on epileptic EEG activity (HVSWs, HPDs, or both) was studied; a group of 12 epileptic FVB/N mice and 7 epileptic NMRI mice was used for this purpose. Final group size analyzed per treatment was 6–8. All mice were used for several treatments; the minimum time interval between two drug experiments in the same animal was at least 4–7 days. Drug injections were always performed at the same time of day, that is, between 9:30 AM and 11:00 AM. As previously reported,¹⁰ administration of drug vehicle had no effect on epileptic EEG activity, so we did not repeat such vehicle experiments. Video/EEG monitoring was started about 24 h prior to each drug experiment. For intraperitoneal drug injections, mice were disconnected from the cables and reconnected immediately afterward. CBZ (Sigma-Aldrich) was administered at doses of 20 and 40 mg/kg i.p., dissolved in 30% polyethylene glycol 400. Dose selection was based on previous studies in the same model^{5,10} and in the 6-Hz mouse model of difficult-to-treat partial seizures.⁹ Respective doses of CBZ are higher than those in traditional seizure models such as the maximal electroshock seizure (MES) test (ED₅₀ of CBZ ~8 mg/kg⁹), because both spontaneous seizures in the intrahippocampal kainate mouse model and seizures in the 6-Hz mouse model are more difficult to suppress than are seizures in the MES test.^{5,9,10} Doses of CBZ exceeding 40 mg/kg were not tested, because the dose inducing “minimal neurological deficit” (or neurotoxicity) in 50% of mice (TD₅₀) in the rotarod test is about 45 mg/kg i.p.⁹

For evaluation of drug effects on epileptic EEG activity, the number and duration of HVSWs and HPDs were visually assessed during four consecutive 0.5-h periods before and after drug injection, respectively, so that each animal was used as its own control. Some additional male NMRI mice were evaluated for effects on HVSWs and HPDs of DZP and phenobarbital (PB), which we recently found to potently suppress HVSWs in female FVB/N mice.¹⁰ DZP was prepared from a commercial ethanol-containing solution (Faustan; Temmler Pharma, Marburg, Germany), which contains 5 mg DZP per ml and was diluted with an

aqueous solution of 5% glucose monohydrate to obtain a DZP dose of 5 mg/kg in an injection volume of 10 ml/kg. PB (Serva, Heidelberg, Germany), 50 mg/kg i.p., was dissolved as sodium salt in saline. The basis for dose selection of PB and DZP was as described above for CBZ.

Statistics

Because the baseline frequency of HVSWs and HPDs varied between the experiments, and because different mouse strains were used in the experiments, we normalized the seizure counts to allow comparisons between different treatments and mouse strains. Thus, HVSWs and HPDs counted in the EEG in the four 0.5-h recording periods before drug treatment were normalized to 100% and compared with the paroxysmal events counted in the EEG in the 2 h after treatment. To avoid missing short-lasting drug effects, the 2 h following treatment were subdivided into four periods of 0.5 h each. For each treatment, the frequency of HVSWs and HPDs following treatment was compared with the seizure frequency before treatment. Data were analyzed by ANOVA, followed by Dunnett's test for post hoc comparisons. A $p < .05$ was considered significant.

RESULTS

Definition of HVSWs and HPDs

For the characterization of HVSWs and HPDs in the mice evaluated here, ~60 h of EEGs from 19 epileptic mice (12 FVB/N, 7 NMRI) were visually analyzed. As reported previously,^{10,19} female FVB/N mice only rarely exhibited HPDs, so only HVSWs were analyzed for the present experiments. In contrast, both HVSWs and HPDs were frequently observed in male NMRI mice.¹⁹ The effect of CBZ in the

intrahippocampal kainate model was previously evaluated by us in female epileptic FVB/N mice,¹⁰ using the parameters described in Table 1. However, when different investigators of our group used these parameters in subsequent experiments, large variation in HVSW counts occurred between investigators, particularly because the interevent interval between the frequently occurring HVSWs was not clearly defined (Table 1). We therefore developed parameters for defining HVSWs and HPDs that led to consistent results when five different investigators examined EEGs of the same epileptic mice. These parameters are shown in Table 2. Based on these criteria, HVSWs in both mouse strains are characterized by sharp waves with high amplitude of at least three times the EEG baseline, have a duration of at least 5 s, have 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 only spikes with an amplitude of <3 times baseline. HVSWs can show either no clear evolution or some evolution in frequency or pattern. Typical HVSWs are shown in Fig. 1A,B. HVSWs did not differ in morphology between FVB/N and NMRI mice, but their frequency was lower in NMRI mice (Table 3).

The main differentiation between HVSWs and HPDs as shown in Table 2 is that (1) if monomorphic, it is classified as HVSW; (2) if polymorphic, it is classified as HPD only if it has evolution to ≥ 5 Hz for ≥ 5 s. HPDs in male NMRI mice typically start with large-amplitude HVSWs, followed by a train of lower-amplitude spikes of at least 5 s of increased frequency (≥ 5 Hz). Similar to HVSWs, an interevent interval of at least 3 s was also used for HPDs. During this interevent interval, there was either no epileptic EEG activity or only spikes with an amplitude of <2 times baseline. A typical HPD is shown in Fig. 2A, illustrating that

Table 2. Newly defined EEG criteria for different types of epileptic EEG activity (events) recorded from the hippocampal focus in epileptic mice of the intrahippocampal kainate model

Type of epileptic EEG activity	Definition	Comments
HVSW	High amplitude (≥ 3 times baseline)	Visual baseline determination
	Rate ≥ 2 spikes per second on average	Mean frequency of discharges in an event (number of spikes divided by duration of event)
	Length ≥ 5 s	Measured from peak of first spike to peak of last spike
	Morphology: monomorphic or polymorphic	HVSWs can show evolution in frequency or pattern but often are rather regular
	Interevent interval ≥ 3 s	Measured from spike peak to spike peak
HPD	Respond to diazepam	Rapid and complete suppression by diazepam (5 mg/kg)
	Usually starts with HVSW-like activity	Spikes ≥ 3 times baseline and rate <5 Hz
	Rate shows evolution with ≥ 5 Hz for ≥ 5 s	Rate: mean frequency of discharges in at least 5 s of the event (≥ 25 spikes in 5 s)
	Lower amplitude (≥ 2 times baseline)	Counts for high-frequency part of the event
	Length 5–20 s (short HPDs) or >20 s (long HPDs)	Measured from peak of first spike to peak of last spike
	Morphology: polymorphic	Clear evolution in frequencies and patterns
Interevent interval ≥ 3 s	Measured from spike peak to spike peak	
Respond to diazepam	Rapid and complete suppression by diazepam (5 mg/kg)	

HVSWs, high-voltage sharp waves; HPDs, hippocampal paroxysmal discharges.

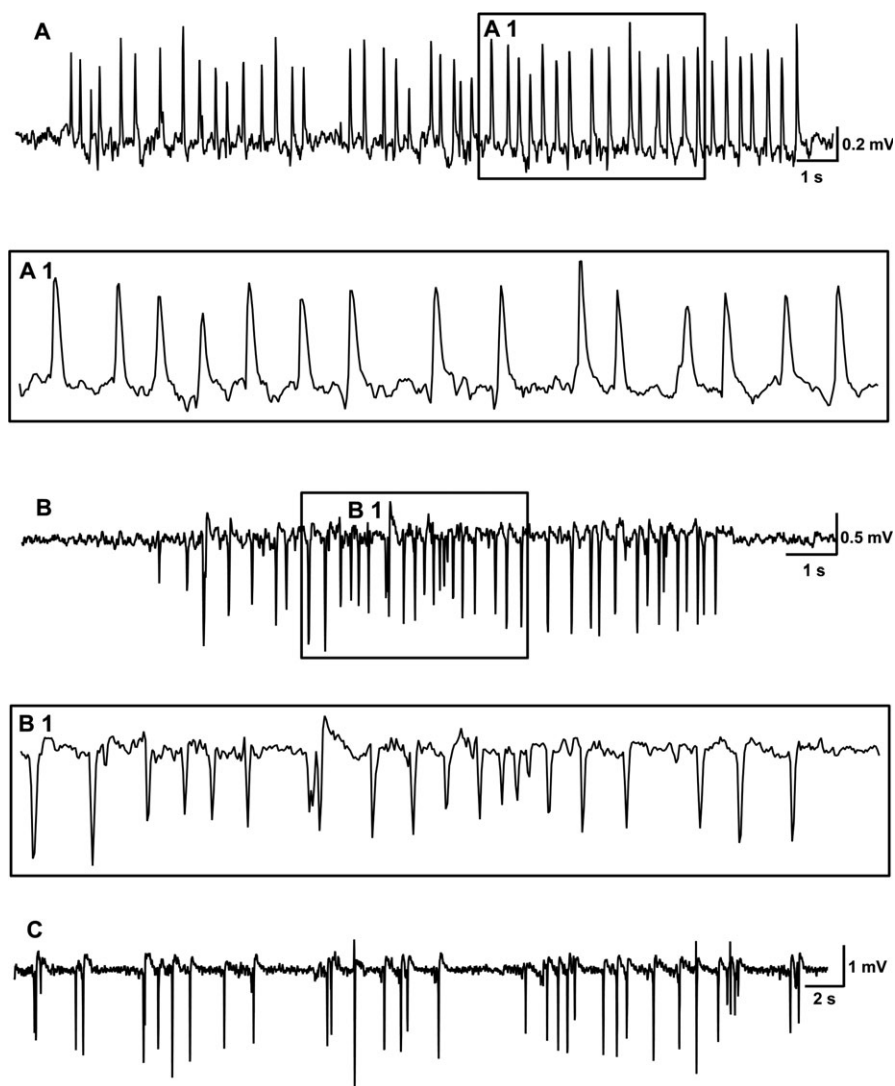


Figure 1.

Representative EEG tracings of chronic epileptic activity recorded from the kainate focus in the ipsilateral hippocampus of epileptic mice. **(A)** A monomorphic high-voltage sharp wave (HVSWS). The boxed area in **A** is enlarged in **A1**, illustrating 5 s of this activity. **(B)** A HVSWS with some evolution in frequency and pattern. The boxed area in **B** is enlarged in **B1**, illustrating 5 s of this activity. **(C)** Typical interictal activity, characterized by irregular appearance of isolated spikes or spike trains (<2 Hz). *Epilepsia Open* © ILAE

HPDs exhibit evolution in morphology and frequency, which is not always seen with HVSWSs. In addition to these typical HPDs, a second type as shown in Fig. 2B was observed. These looked like a mixed event starting with HPD-like activity but then evolving into HVSWS-like activity. This type of mixed activity was assigned to HPDs when we counted HVSWSs and HPDs. In NMRI mice, HPDs occurred about 7–10 times per hour on average compared to 13–16 HVSWSs per hour (Table 3). During direct observation of epileptic mice or in the videos recorded during hippocampal HVSWSs and HPDs, no clear behavioral alterations were seen, but subtle alterations may have been overlooked. In addition to HVSWSs and HPDs, interictal EEG activity was seen in all epileptic mice. Interictal activity was characterized by irregular appearance of isolated spikes or spike trains (<2 Hz) as shown in Fig. 1C. This activity was not further analyzed in the drug studies described below.

Effect of HVSWS definition on HVSWS frequency and the effect of CBZ on HVSWS frequency

For direct evaluation of how the definition of HVSWSs affects HVSWS frequency and the effect of CBZ on HVSWSs, HVSWSs were counted in the same mice by either the HVSWS definition previously used by Klein et al.¹⁰ (see Table 1) or the new HVSWS definition as shown in Table 2. A dramatic difference in HVSWS frequency was found (Table 3). Average HVSWS frequency in two experiments in FVB/N mice was 108 and 171 HVSWSs per hour with the previous definition compared to 19 HVSWSs per hour (in both experiments) with the new definition ($p < .0001$). This marked difference was mainly because of the higher frequency (≥ 2 Hz) used for inclusion of HVSWSs by the new method so that many HVSWSs with lower frequency included by the old method were not counted. Furthermore, the newly defined interevent interval and minimum duration of HVSWSs added to the counting differences between the old and new methods.

Table 3. Frequency of epileptic EEG events in the predrug control recordings in female FVB/N and male NMRI mice. Frequencies were determined either by the “old method” as described by Klein et al.¹⁰ or the “new method” described in this study (see Table 1). Note that epileptic EEG events in female FVB/N mice were almost exclusively HVSWs, whereas both HVSWs and HPDs occurred in male NMRI mice. All data are given as mean and range

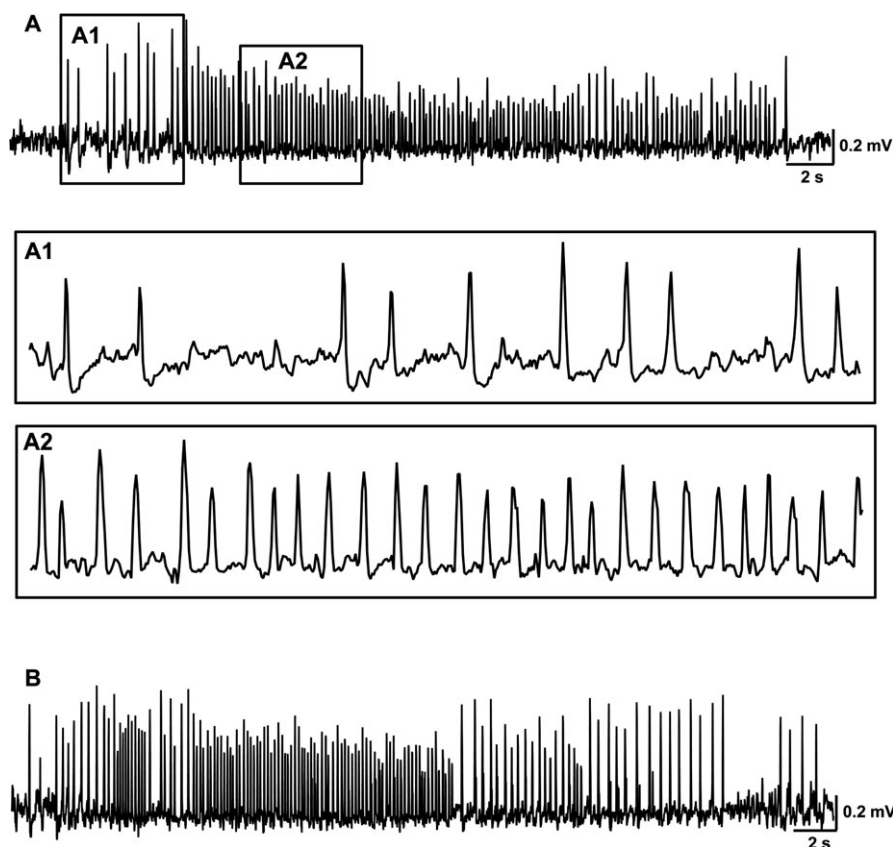
	Frequency of epileptic EEG events (not further differentiated into HVSWs and HPDs) during predrug control recordings		Frequency of HVSWs during predrug control recordings	Frequency of HPDs during predrug control recordings
	Old method	New method		
FVB/N mice				
Predrug 20 mg/kg CBZ (n = 8)	108 (80–151) per h	19 (1–37) per h*		
Predrug 40 mg/kg CBZ (n = 8)	171 (149–232) per h	19 (5–36) per h*		
NMRI mice				
Predrug 20 mg/kg CBZ (n = 6)	78 (52–115) per h	23 (8–38) per h*	13 (2–28) per h	10 (0–17) per h
Predrug 40 mg/kg CBZ (n = 6)	102 (44–177) per h	23 (6–38) per h*	16 (6–24) per h	7 (0–16) per h

CBZ, carbamazepine; HVSWs, high-voltage sharp waves; HPDs, hippocampal paroxysmal discharges.
The new method resulted in significantly fewer epileptic EEG events than the old method, which is indicated by asterisk ($p < .01$).

Figure 2.

Representative EEG tracings of chronic epileptic activity recorded from the kainate focus in the ipsilateral hippocampus of epileptic mice. **(A)** A polymorphic hippocampal paroxysmal discharge (HPD). Most HPDs start with HVSW-like activity, followed by increased spike frequency but lower spike amplitude compared to HVSWs. The boxed areas in **A** are enlarged in **A1** and **A2**, illustrating 5 s of this activity for each box. **(B)** An HPD that looks like a mixed HPD/HVSW event, starting with typical high-frequency (≥ 5 Hz) HPD activity, but then evolution to a slower pattern of HVSW-like activity occurs. Such events were assigned to HPD-like activity.

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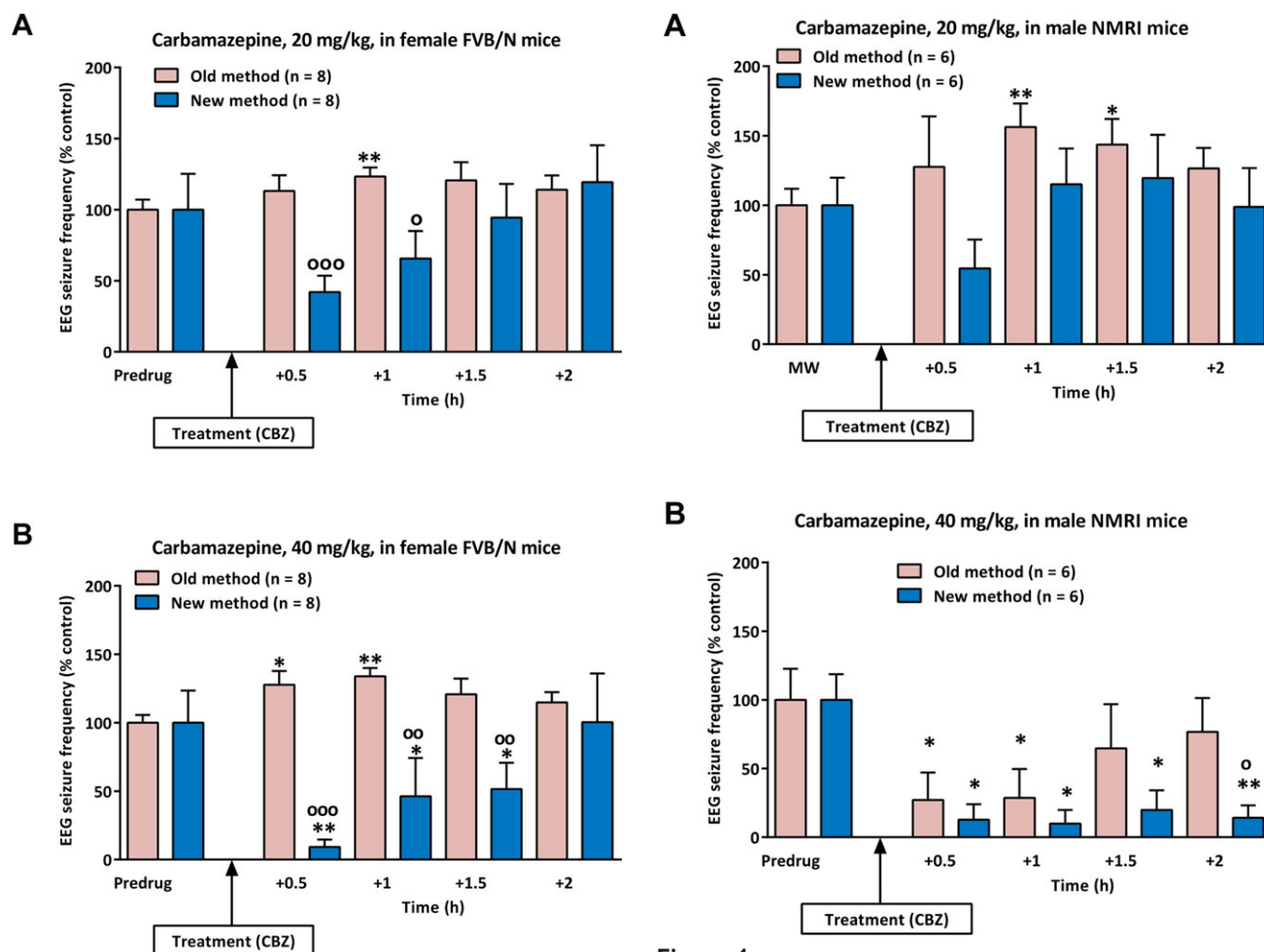


The new definition of HVSWs also had a striking effect on the response to CBZ. As shown in Fig. 3, 20 or 40 mg/kg CBZ did not suppress HVSWs defined by the old method (as used in the Klein et al.¹⁰ study) but instead exerted a pro-convulsant effect as reported previously.¹⁰ In contrast, when using the new HVSW definition, CBZ tended to decrease HVSWs at 20 mg/kg ($p = .0692$), and a significant reduction of HVSWs was determined at 40 mg/kg (Fig. 3B).

Thus, just by altering the inclusion criteria for HVSWs, a qualitative difference in drug response was obtained.

Effect of mouse strain on the effect of CBZ in the intrahippocampal kainate model

As in the previous studies of Maroso et al.⁷ with PHT, we started CBZ experiments in male NMRI mice by testing the effect of CBZ on both types of epileptic activity, HVSWs

**Figure 3.**

Effect of carbamazepine (CBZ) on epileptic EEG activity in FVB/N mice. 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 compared with two different definitions of epileptic activity (see text), the “old” method used previously by us as described by Klein et al.¹⁰ (see Table 1) and the “new” method described in Table 2. Epileptic activity analyzed by the two methods almost completely consisted of high-voltage sharp waves (HVSWs), whereas hippocampal paroxysmal discharges (HPDs) were rarely seen in female FVB/N mice. HVSWs counted in the EEG in the 2 h before drug treatment were normalized to 100% and compared with the HVSWs counted in the EEG in the 2 h after treatment. See Table 3 for absolute counts in the predrug period. **(A)** The effect of 20 mg/kg CBZ and **(B)** the effect of 40 mg/kg i.p. Data are shown as mean \pm SEM of eight mice per group. Statistical differences between predrug control and postdrug values within each group are indicated by asterisk (* $p < .05$; ** $p < .01$), whereas statistical differences between data analyzed by the old versus new method are indicated by circle ($^{\circ}P < .05$; $^{\circ\circ}P < .01$; $^{\circ\circ\circ}P < .0001$). Note that in **A** there was a tendency for an antiseizure effect of CBZ (calculated by the new method) already at 20 mg/kg ($p = .0692$).

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Figure 4.

Effect of carbamazepine (CBZ) on epileptic EEG activity in epileptic NMRI mice. 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 compared with two different definitions of epileptic activity (see text), the “old” method used previously by us as described by Klein et al.¹⁰ (see Table 1) and the “new” method described in Table 2. Epileptic activity analyzed by the two methods in NMRI mice consisted of high-voltage spike waves (HVSWs) and hippocampal paroxysmal discharges (HPDs), which were summed up for this analysis. EEG seizure frequency counted in the 2 h before drug treatment was normalized to 100% and compared with seizure frequency counted in the EEG in the 2 h after treatment. See Table 3 for absolute HVSW and HPD counts in the predrug period. **(A)** The effect of 20 mg/kg CBZ and **(B)** the effect of 40 mg/kg i.p. Data are shown as mean \pm SEM of six mice per group. Statistical differences between predrug control and postdrug values within each group are indicated by asterisk (* $p < .05$; ** $p < .01$), whereas statistical differences between data analyzed by the old versus new method are indicated by circle ($^{\circ}P < .05$).

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and HPDs, together so that the data in Fig. 4 do not differentiate between different types of spontaneous EEG epileptic activity. This also allows comparing CBZ's effects in female FVB/N (Fig. 3) and male NMRI (Fig. 4) mice. Furthermore, the two methods (old vs. new) of defining epileptic activity in FVB/N mice were also used in NMRI mice. As shown in Fig. 4A, at 20 mg/kg, the effects of CBZ in NMRI mice were similar to those obtained in FVB/N mice in that no significant suppression of epileptic activity was observed. However, at 40 mg/kg, CBZ significantly suppressed epileptic activity irrespective of the seizure definition used (Fig. 4B). This pronounced antiseizure effect of CBZ lasted for at least 2 h. Thus, male NMRI mice (Fig. 4) were more sensitive to the antiseizure effect of CBZ than were female FVB/N mice (Fig. 3).

The effects of CBZ on frequency of HVSWs and HPDs differ

Next, we differentiated the spontaneous epileptic EEG activity of NMRI mice into HVSWs and HPDs using the new criteria as outlined in Table 2. As shown in Fig. 5A, at 20 mg/kg, CBZ significantly suppressed HPDs, but not HVSWs. Thus, at this dose, CBZ changed the relative proportion of the two electrographic endpoints (i.e., HVSWs and HPDs). At 40 mg/kg, both types of paroxysmal EEG activity were markedly suppressed (Fig. 5B).

Effects of CBZ on the duration of HVSWs and HPDs

In addition to evaluating the effect of CBZ on frequency of spontaneous epileptic EEG activity, we analyzed the effect of CBZ on cumulative duration of this activity. The newly defined EEG criteria shown in Table 2 were used for calculating cumulative duration of epileptic EEG events (HVSWs and HPDs). When cumulative duration of epileptic EEG activity was analyzed for the 2 h before and after CBZ administration, CBZ did not exert any significant effect at 20 mg/kg in the two mouse strains, while significant effects were observed at 40 mg/kg (Fig. 6A,B). When the cumulative duration was separately calculated for HVSWs and HPDs in NMRI mice, effects of CBZ resembled those observed for frequency of these events (Fig. 5) in that at 20 mg/kg no significant effect on HVSWs was observed, while cumulative duration of HPDs was significantly reduced (Fig. 6C). At 40 mg/kg CBZ, cumulative duration of both HVSWs and HPDs was markedly reduced (Fig. 6D).

Interestingly, when cumulative duration of epileptic activity recorded during the 2-h predrug control periods was compared between FVB/N and NMRI mice, it was significantly higher in NMRI (30.7 ± 5.6 min/2 h) than FVB/N mice (17.9 ± 3.3 min/2 h; $p = .0474$). None of the FVB/N mice had a series of recurrent independent electrographic seizures totaling more than 30 min in any 1-h period (50% seizure burden) during predrug control recordings; this was observed in 2 NMRI mice and blocked by 40 mg/kg CBZ (Fig. 6B). We did not observe any obvious changes in the

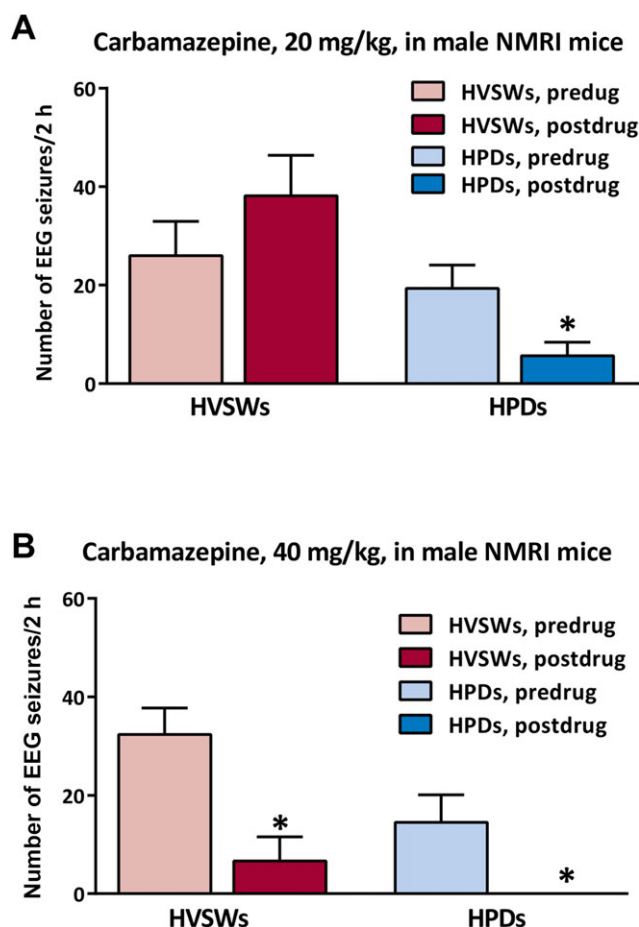


Figure 5. Effect of carbamazepine (CBZ) on epileptic EEG activity in epileptic NMRI mice. Epileptic activity was recorded from an electrode located at the kainate injection site in the CA1 region of the dorsal hippocampus. The effect of CBZ was separately assessed for high-voltage spike waves (HVSWs) and hippocampal paroxysmal discharges (HPDs). (A) The effect of 20 mg/kg CBZ and (B) the effect of 40 mg/kg i.p. Data are shown as mean \pm SEM of six mice per group and were recorded in the 2 h before and after CBZ. Statistical differences between predrug control and postdrug values within each group are indicated by asterisk (* $p < .05$; ** $p < .01$). *Epilepsia Open* © ILAE

morphology of the electrographic events by CBZ in any mouse strain, although this was not systematically analyzed.

Diazepam and phenobarbital block both HVSWs and HPDs

For comparison with the effects of CBZ, some experiments were also performed with DZP and PB in male NMRI mice. As shown for a representative mouse in Fig. 7A,B, DZP, 5 mg/kg i.p., rapidly and completely suppressed both HVSWs and HPDs, which was confirmed in additional mice. To examine whether long HVSWs and HPDs were more difficult to treat than shorter ones, HVSWs and HPDs

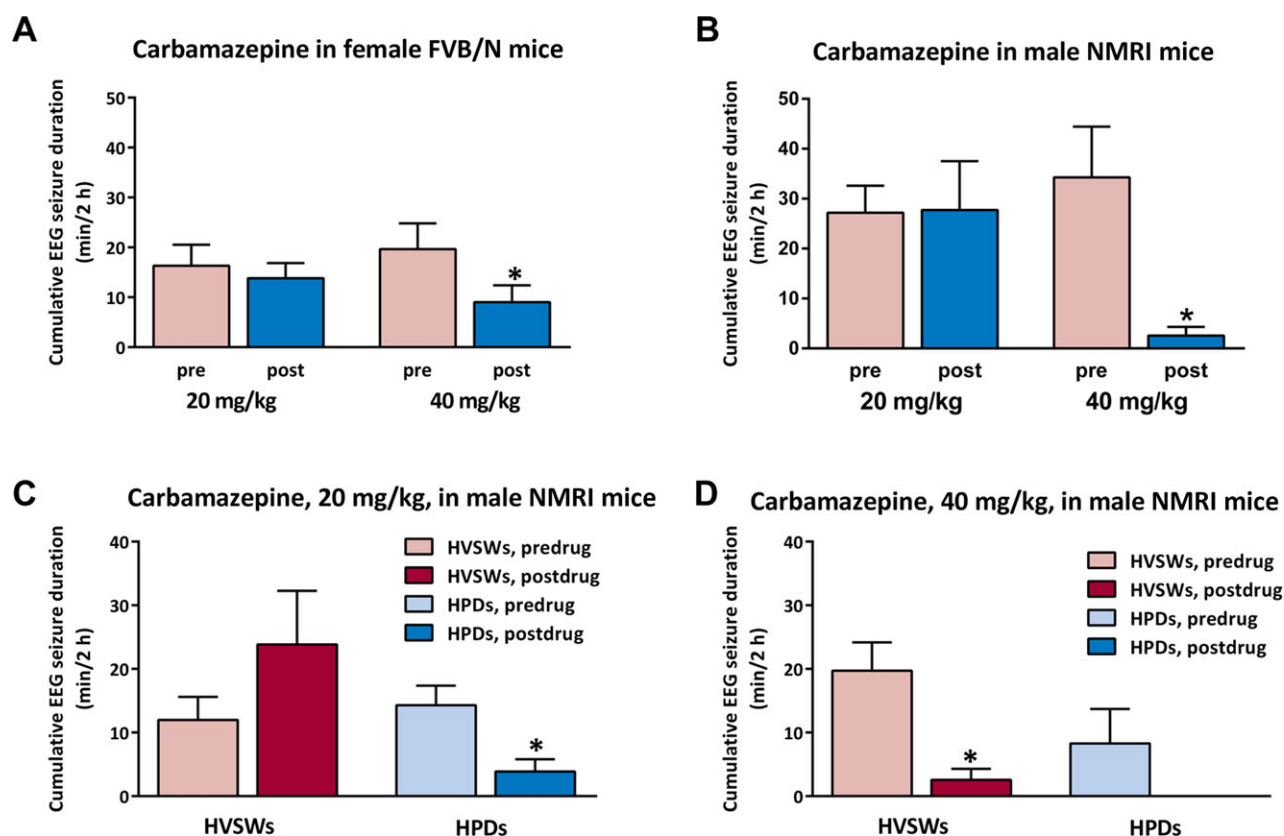


Figure 6.

Effect of carbamazepine (CBZ) on cumulative duration of electrographic seizures in FVB/N (**A**) and NMRI (**B–D**) mice. In **A** and **B**, cumulative EEG seizure duration is not further differentiated into high-voltage spike waves (HVSWs) and hippocampal paroxysmal discharges (HPDs), whereas **C** and **D** show data separately for HVSWs and HPDs in NMRI mice. All data are shown as cumulative EEG seizure duration (mean \pm SEM) over 2 h before (pre) and after (post) administration of CBZ in groups of 8 (FVB/N) and 6 (NMRI) mice, respectively. Statistical differences between predrug control and postdrug values within each group are indicated by asterisk (* $p < .05$).

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are grouped with respect to length in Fig. 7. PB (50 mg/kg) had a similar pronounced effect on HVSWs and HPDs as DZP (Fig. 7C,D), but the onset of HVSW suppression was moderately retarded.

Tolerability of drug treatments

At 20 mg/kg, CBZ was tolerated without any obvious behavioral adverse effects. At 40 mg/kg, sedation and ataxia were observed, which could be expected, because the TD_{50} of CBZ in the rotarod test is about 45 mg/kg i.p.⁹ Following injection of DZP, 5 mg/kg, mice exhibited reduced locomotor activity and moderate sedation. PB, 50 mg/kg, was tolerated without any obvious behavioral alterations except moderate hyperactivity for about 20 min (TD_{50} of this drug in mice is about 70 mg/kg i.p.⁹).

DISCUSSION

There is an ongoing debate about the fundamental definition of a seizure, both in the clinic and in experimentally

used rodents.^{3,20–24} According to the definition of the International League Against Epilepsy (ILAE), seizures are defined as a “transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity of the brain.”²⁵ Including symptoms and signs in the definition of a clinical seizure is indispensable but leaves subclinical (electrographic) seizures poorly defined.²³ Furthermore, there is uncertainty how to distinguish between interictal and ictal EEG activity.²² For nonconvulsive seizures recorded in the intensive care unit, a number of EEG criteria have been described, including 3-Hz or faster periodic discharges and a definite evolution in morphology, location, or frequency, to distinguish on only the basis of the EEG pattern whether the EEG pattern is unequivocally ictal or seizure based (Table 4).²⁶ Furthermore, clinically a duration of at least 10 s is used as an arbitrary cutoff for nonconvulsive seizures; seizures or potential seizures that are less than 10 s are called B(I)RDs (brief potentially ictal rhythmic discharges).²⁶ However, it is not clear whether these definitions can be directly transferred to animal models.

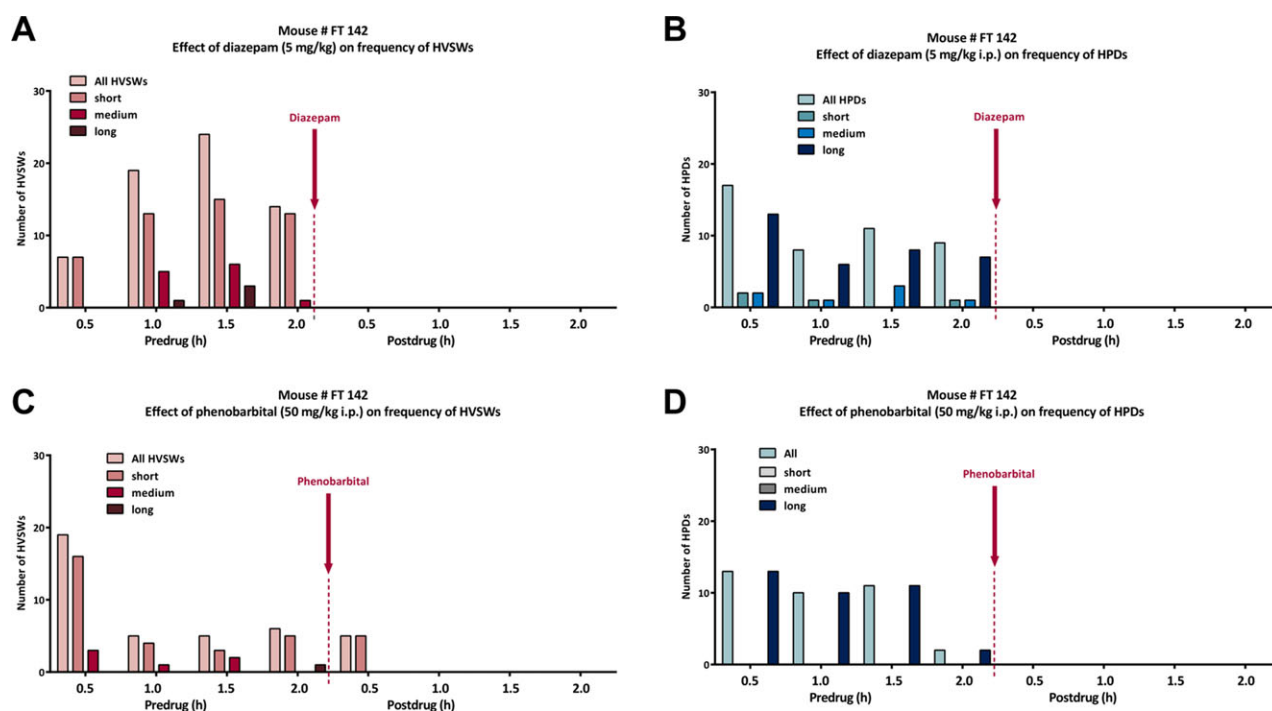


Figure 7.

Illustration of two experiments in one male epileptic NMRI mouse in which either diazepam (**A, B**) or phenobarbital (**C, D**) was administered after 2 h of predrug control EEG recording. Epileptic activity was recorded from an electrode located at the kainate injection site in the CA1 region of the dorsal hippocampus. The antiseizure effect of the two drugs is separately shown for high-voltage sharp waves (HVSWs; **A, C**) and hippocampal paroxysmal discharges (HPDs; **B, D**). Both types of electrographic seizures were subdivided into short (≥ 3 s and < 10 s), medium (≥ 10 and < 20 s), and long (> 20 s) events. *Epilepsia Open* © ILAE

Furthermore, symptoms associated with epileptic EEG alterations in animals may be so subtle that they are not easily recognized. Examples are behavioral arrest, increased whisker twitching and chewing, and stereotyped behaviors, such as exploration, grooming, and scratching, which can represent the only symptoms of limbic seizures in rodents.^{27,28} Furthermore, paroxysmal events in the hippocampus, as described in the present study, may induce a memory lapse that cannot be easily detected in an animal.²²

As described earlier, in the intrahippocampal kainate mouse model, Riban et al.⁵ characterized two types of chronic epileptic activity in the EEG recorded from the ipsilateral hippocampus, that is, HVSWs and HPDs (Table 1). Whereas HVSWs were not associated with behavioral alterations, HPDs, which occurred at a frequency of up to 60/h, were often associated with behavioral arrest, head nodding, or stereotyped behavior, such as exploration or grooming. HVSWs could sometimes also be recorded with some delay (8–12 ms) in the contralateral hippocampus (but never in the cerebral cortex), whereas the HPDs were observed only in the ipsilateral hippocampus (within 0.5 mm from the kainate injection site) and not contralaterally or in cerebral cortex.⁵ Riban et al.⁵ suggested that HVSWs may be considered interictal events that are dissociated from seizures but that may initiate HPDs, whereas HPDs are focal

nonconvulsive seizures resembling hypersynchronous high-voltage spikes observed in sclerotic hippocampus of patients with TLE, particularly when this structure is the focus of epileptic activity. Riban et al.⁵ reported that both HVSWs and HPDs were suppressed by DZP, whereas HPDs were resistant to CBZ, PHT, and VPA. In contrast to the frequent HPDs and HVSWs, generalized convulsive seizures were only rarely observed.⁵

In a subsequent study by Maroso et al.,⁷ the findings of Riban et al.⁵ in Swiss mice were confirmed by using male C57BL/6 mice (Table 1). Maroso et al.⁷ reported that HPDs represent ~35% of chronic paroxysmal EEG activity in this model and typically start with large-amplitude sharp waves (1–3 mV; 1–3 Hz) followed by a train of spikes of increasing frequency (0.5–1.0 mV; 10–20 Hz) and terminate with a deflection in the EEG. PHT did not suppress HPDs and HVSWs, which were counted together for drug experiments.⁷ Both types of paroxysmal EEG alterations were considered epileptic activity, whereas interictal activity was defined as isolated spikes or spike trains with a frequency of 1–3 Hz and a duration of < 20 s.⁷

Surprisingly, when we established the intrahippocampal kainate model in female FVB/N mice, the predominant epileptic activity in the EEG was HVSWs (Table 1), whereas HPDs and convulsive seizures were only rarely

Table 4. EEG criteria for nonconvulsive seizures (NCSs) in patients in intensive care units (from Sinha and Hirsch²⁶) and observations in the present study in the intrahippocampal kainate mouse model. Note that NCSs need only any one of the three primary criteria

EEG criteria for nonconvulsive seizures (NCSs) in patients	EEG criteria fulfilled by epileptic EEG activity in the intrahippocampal kainate mouse model	
	HVSWs	HPDs
Primary criteria		
1. Repetitive generalized or focal epileptiform discharges at a rate of ≥ 3 Hz	Possible (see Table 2)	Yes
2. Repetitive generalized or focal epileptiform discharges at a rate of < 3 Hz and the secondary criterion	Yes	
3. Sequential rhythmic, periodic, or quasiperiodic waves at ≥ 1 Hz and unequivocal evolution in frequency (gradually increasing or decreasing by at least 1 Hz), morphology, or spatial extent. Excludes evolution in amplitude alone or change in sharpness alone	Possible (see Table 2)	Possible (see Table 2)
Secondary criterion		
Significant improvement in clinical state or appearance of previously absent normal EEG patterns in response to acute administration of a rapid-acting AED, such as a benzodiazepine	Yes (for 5 mg/kg DZP)	Yes (for 5 mg/kg DZP)
Definition of evolution		
1. Frequency: at least two consecutive changes in the same direction by at least 0.5/s, e.g., from 2 to 2.5 to 3/s, or from 3 to 2 to 1.5/s	Possible (see Table 2)	Possible, but at least one change (see Table 2)
2. Morphology: at least two consecutive changes to a novel morphology	Possible (see Table 2)	Possible, but at least one change (see Table 2)
3. Location: sequential spreading into or sequentially out of at least two different standard 10–20 electrode locations	Not applicable	Not applicable
To qualify as present, a single frequency or location must persist for at least three cycles. The criteria for evolution must be reached without the pattern remaining unchanged in frequency, morphology, or location for ≥ 5 min	No (for specific definitions of frequency and morphology see Table 2)	
Duration of NCS		
Ten seconds is used as an arbitrary cutoff; NCSs or potential NCSs shorter than 10 s are called brief potentially ictal rhythmic discharges (B[]RDs)	Five seconds is used as an arbitrary cutoff; spike trains or discharges < 5 s are regarded as interictal activity	
AED, antiepileptic drug; DZP, diazepam; HVSWs, high-voltage sharp waves; HPDs, hippocampal paroxysmal discharges; NCS, nonconvulsive seizure. It is important to note that these are criteria for definite NCSs. If a pattern does not fulfill these criteria, it does not mean that NCSs have been ruled out; it only means that NCSs cannot be ruled in definitively.		

observed.¹⁰ HVSWs were resistant to CBZ and PHT, whereas both ASDs blocked secondarily generalized seizures,¹⁰ which is consistent with clinical experience that ASDs often limit the generalization of seizures while their effects on focal seizures may be less pronounced.³ In a subsequent study, in which we compared the model in male and female NMRI mice, frequent HPDs were observed only in male mice, whereas both sexes exhibited frequent HVSWs (~20–30/h) and infrequent convulsive seizures.¹⁹ Because of the uncertainty about whether HVSWs are ictal or interictal events, we used the term “seizure-like event” (SLE) in our previous studies, a term that is commonly used for describing paroxysmal activity in *in vitro* preparations such as the hippocampal slice²⁹ but that is only rarely used in *in vivo* models.

As shown in Table 4, HPDs fulfill the clinical criteria of nonconvulsive (electroclinical) seizures when associated with behavioral changes or electrographic seizures when

obvious behavioral correlates are not observed as in the present study. They show clear evolution in frequency and pattern and some temporal duration. HVSWs often lack any clear evolution but are rather monomorphic paroxysmal bursts. This, however, does not argue against the possibility that they represent electrographic seizures. Monomorphic focal EEG seizures are not uncommon in patients with different types of epilepsy^{30–32} and have also been described in other animal models of acquired epilepsy, such as the perinatal hypoxia model of epilepsy.^{33,34} Furthermore, the fact that HVSWs can be suppressed by rapidly acting ASDs such as DZP as shown by Klein et al.¹⁰ in FVB/N mice and here for NMRI mice would be consistent with a nonconvulsive seizure definition as shown in Table 4.

The finding that both HPDs and HVSWs could be suppressed by CBZ in male NMRI mice was unexpected, because previous studies reported that both types of electrographic seizures are resistant to CBZ when administered at

doses of 20–50 mg/kg in male and female mice of different strains, including NMRI.^{5,6,10,35} A similar effect of genetic background on resistance to an ASD (PHT) acting by modulation of sodium channels has recently been reported for the 6-Hz mouse model of difficult-to-treat partial seizures.⁸ In this model, in which partial seizures are induced by corneal stimulation with a 6-Hz current of different current intensities,⁹ male C57BL/6 mice were nearly completely resistant to PHT, whereas male NMRI mice responded well to PHT.⁸ Similar findings were reported for levetiracetam.⁸ The unexpected interstrain difference in antiseizure efficacy was not due to pharmacokinetic differences or differences in seizure threshold.⁸ Thus, the data of Leclercq and Kaminski⁸ and the present data demonstrate that treatment resistance in mouse models such as the intrahippocampal kainate and 6-Hz models should be interpreted with the genetic background of mice in mind. Furthermore, as shown recently, the sex of the mice has an effect on epilepsy models because, similar to female FVB/N mice, female NMRI mice do only rarely exhibit HPDs in the intrahippocampal kainate mouse model.¹⁹ Thus these models are more complex than previously thought.

In the present study we compared the antiseizure efficacy of CBZ in female FVB/N and male NMRI mice because we had previously characterized the pharmacological response of HVSWs in female FVB/N mice¹⁰ and so could use these previous data as a basis for dose selection for the comparison with male NMRI mice. We cannot exclude that sex differences alone may introduce potential confounds in interpretation of the relative efficacy/inefficacy of CBZ. One may thus argue that in the absence of a head-to-head sex comparison, it is not clear how generalizable the findings are. Therefore, gender differences in the pharmacological response of these mouse strains should be further explored. However, the marked antiseizure effect of 40 mg/kg CBZ observed in the present study in male NMRI mice is clearly in contrast to the previously reported resistance of male mice of other strains to CBZ,^{5,35} thus demonstrating a clear strain difference. Furthermore, the data of Leclercq and Kaminski,⁸ in which the pharmacological response of the 6-Hz model of difficult-to-treat partial seizures was compared in male NMRI, C57BL/6, and CF-1 mice, showed that male NMRI mice were much more sensitive to ASDs in this model than the two other mouse strains, which is in line with the present findings in epileptic NMRI mice.

In a previous study in male NMRI mice, Gouder et al.⁶ reported that 30 mg/kg CBZ was not capable of suppressing HPDs in the intrahippocampal kainate mouse model, whereas HPDs were suppressed by an adenosine A₁-receptor agonist. In the present study, both 20 and 40 mg/kg CBZ suppressed HPDs in male NMRI mice. The major difference between this previous and the present study was the vendor (Harlan vs. Charles River) from which the outbred NMRI mice were obtained. Outbred mouse strains such as NMRI are widely used in many fields of research, including

pharmacology.¹¹ Such mice are randomly outbred; hence, allelic variations can occur across separate colonies so that outbred mice from different vendors may have little in common with each other besides their names and similarities in pelage.³⁶ Substrain differences in NMRI mice are the most likely explanation for the differences between previous and present experiments with CBZ in the intrahippocampal kainate mouse model and add to the complexity of this model.

In addition to mouse strain or substrain differences in ASD activity in the intrahippocampal kainate model, we found that the criteria used to define epileptic EEG activity have a striking effect on ASD efficacy. In our previous study in female FVB/N mice,¹⁰ we found that CBZ and PHT were not capable of suppressing paroxysmal EEG events, mostly HVSWs, which was consistent with previous studies on these drugs in the intrahippocampal kainate model.^{5–7} Instead, both CBZ and PHT increased HVSW frequency, indicating proconvulsant activity.¹⁰ However, by using stricter criteria for defining HVSWs (to distinguish them from interictal spikes), including an inter-HVSW interval that was not clearly defined in previous studies,^{5–7,10} we found that the effect of CBZ on epileptic EEG events strikingly changed so that FVB/N mice were not resistant anymore. Similarly, CBZ suppressed both HVSWs and HPDs in NMRI mice. Interestingly, HVSWs were more difficult to suppress by CBZ than were HPDs in NMRI mice.

How can this qualitative difference in CBZ's effects on differently defined HVSWs be explained? According to the previously used criteria for HVSW definition, both doses of CBZ in FVB/N mice and the lower dose (20 mg/kg) in NMRI mice exerted proconvulsant activity, whereas antiseizure effects were obtained when using the newly defined HVSW criteria. For the latter criteria, in addition to defining an inter-HVSW interval, which lowers the HVSW number by fusing two HVSWs into one, we also increased the minimum frequency from ≥ 1 Hz¹⁰ to ≥ 2 Hz. Thus, HVSWs with a frequency of < 2 Hz were ignored in HVSW counting. This resulted in a marked decrease in HVSW counts from ~ 100 /h to ~ 20 /h, indicating that most HVSWs counted previously had a frequency of < 2 Hz. As in patients,²⁶ rodents may also have an ictal-interictal continuum with no real dichotomy between ictal and interictal so that it appears likely that HVSWs < 2 Hz are interictal activity that does not respond well to ASDs such as CBZ or PHT,¹⁰ whereas HVSWs ≥ 2 Hz are paroxysmal events that can be blocked by CBZ (as well as DZP and PB) as shown here.

The major aim of developing robust criteria for HVSW/HPD counting in the EEG was to reduce the variation among investigators analyzing the same EEGs, which was observed with less rigid criteria. Another aim was to use these criteria for automatic detection of paroxysmal events in the EEG. First experiments with an EEG data acquisition software (DataWave Technologies, Longmont, Colorado) indicate that it is possible to analyze paroxysmal EEG

events in epileptic mice and drug effects on such events with high sensitivity and selectivity; however, it was not possible to differentiate HVSWs and HPDs by this program (Vladan Rankovic; unpublished data). We currently are trying to develop an algorithm that allows separate detection of HVSWs and HPDs.

When we recorded the EEG via a hippocampal electrode in sham FVB/N controls, short bursts of HVSW-like activity were observed in some animals, but at much lower frequency than in kainate-treated mice.¹⁰ However, this does not imply that HVSWs represent normal EEG events, because the depth electrode in the hippocampus might induce proepileptogenic effects by itself, most likely as a result of blood–brain barrier disruption resulting in extravasation of albumin, local microhemorrhages, or inflammation.^{3,37–39} In control mice with skull electrodes not penetrating into the brain, we did not observe any paroxysmal EEG activity (K. Töllner, W. Löscher, unpublished observations). Similar to the observation in sham control mice with depth electrodes reported by Klein et al.,¹⁰ short SLEs (2–10 s) characterized by sharp waves have also been observed in control rats with depth electrodes.⁴⁰ However, such EEG events in sham controls were only observed in rats at 7–8 months of age and not in younger rats.⁴⁰ Similarly, intermittent low-frequency epileptiform discharges were recorded via hippocampal depth electrodes in control rats.^{33,34} However, as in the studies in mice, these SLEs in rats were clearly different in several aspects, including morphology, incidence, and frequency, from those in models of acquired epilepsy in rats.^{33,34,40} In addition to the possibility that such SLEs in sham controls are a consequence of depth-electrode-induced lesions, inherent 8- to 11-Hz spike-wave discharges (SWDs) are commonly recorded in both inbred and outbred rat strains, but these SWDs are easily distinguished from lesion-induced ictal discharges in models of traumatic brain injury.⁴¹ Spontaneous SWDs (or other types of nonconvulsive seizures) do not occur in most inbred and outbred mouse strains, including those used in the present study,⁴² but 6- to 8-Hz SWDs have been described in some inbred strains such as DBA/2, C3H/HeJ, and A/J.^{42–44} Interestingly, in male sham NMRI control mice with depth electrode in the hippocampus, no HVSW- or HPD-like EEG activity was observed but only some infrequent spikes (F. Twele, unpublished data). An ILAE/American Epilepsy Society (AES) 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.⁴⁵

In patients with TLE, seizures are difficult to define electroencephalographically.⁴⁶ Seizures with clinical symptoms are often only a small proportion of all abnormal electrical activity in the brain, which includes subclinical seizures, interictal spikes, bursts, and high-frequency oscillations.⁴⁶ CBZ has been reported to increase interictal spiking in

patients and animal models of TLE,^{47–49} but this was not systematically evaluated in the present study in the intrahippocampal kainate mouse model of TLE. In a recent study in this model, Duveau et al.³⁵ reported that high doses of CBZ (>50 mg/kg) increase the occurrence of interictal spikes. In the latter study, also ictal and interictal power spectra were analyzed, showing an increased power of interictal beta oscillations and an increased amplitude and power of residual HPDs at a dose of CBZ (75 mg/kg i.p.) that significantly reduced the cumulative duration of HPDs.

In the present study, CBZ doses exceeding 40 mg/kg were avoided, because ataxia and sedation become increasingly severe at higher doses of this drug, resulting in a TD₅₀ in the rotarod test in mice of about 45 mg/kg.⁹ Pharmacoresistance in animal models has been operationally defined as persistent seizure activity that does not respond to monotherapy at tolerable doses with at least two current ASDs.⁵⁰ Thus, antiseizure efficacy of ASDs such as CBZ and of other ASDs at high neurotoxic doses as reported by Duveau et al.³⁵ in the intrahippocampal kainate mouse model are difficult to interpret. On the basis of previous pharmacokinetic analyses in mice,⁵¹ plasma concentrations achieved at time of maximum antiseizure effect of CBZ (15 min) at the doses used in the present study would be 7.3 µg/ml (20 mg/kg) and 14.5 µg/ml (40 mg/kg), which is closely related to the “therapeutic plasma concentration range” (4–12 µg/ml) in patients with epilepsy.⁵² The pharmacokinetic analyses also showed that, because of rapid elimination, the duration of the antiseizure effect in mice is only about 2 h,⁵¹ which was also found in the present experiments.

The present data further substantiate that the intrahippocampal mouse model is unique because of its high frequency of electrographic (or nonconvulsive) seizures that do not occur at such high frequency in any other model of TLE,³ including the intrahippocampal kainate model in rats.⁵³ Both HPDs and HVSWs may occur at such high frequency in mice (Table 1) that they might be considered as electrographic SE (ESE), as, for instance, occurring in children with acute neurologic disorders and contributing to secondary brain injury and worse short-term and long-term outcomes.⁵⁴ In patients, ESE is defined as either a single 30-min electrographic seizure or a series of recurrent independent electrographic seizures totaling more than 30 min in any 1-h period (50% seizure burden).⁵⁴ The total duration of electrographic seizures calculated per hour in the present study in the mouse model during control periods did not identify any epileptic mouse meeting the definition of ESE in the FVB/N strain, whereas two epileptic NMRI mice exhibited a cumulative EEG seizure duration of >30 min/h during predrug control, which was blocked by CBZ (40 mg/kg).

In conclusion, similar to recent observations in the 6-Hz mouse model,⁸ the present data demonstrate that focal seizures in the intrahippocampal kainate mouse model are less

pharmacoresistant than previously thought. Indeed, both mouse (sub)strain differences and the criteria chosen for definition of seizures in the EEG determine whether such seizures are responsive to ASDs. Although the present mouse strain comparisons were restricted to the major ASD CBZ, it is very likely that the observations reported here can be extended to other ASDs. Taken together, these converging observations highlight an important methodological issue, which has not been previously taken into consideration. Intriguingly, however, these results appear to have high translational value because response or resistance to ASDs in patients with epilepsy is likely to have genetic underpinnings in addition to the well-established genetic susceptibility to epilepsy. Ultimately, such models can now be used to study potential genetic variance governing drug response as opposed to mere efficacy testing.

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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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