



RESEARCH ARTICLE

Preclinical efficacy profiles of the sigma-1 modulator E1R and of fenfluramine in two chronic mouse epilepsy models

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Abstract

Objective: Given its key homeostatic role affecting mitochondria, ionotropic and metabotropic receptors, and voltage-gated ion channels, sigma-1 receptor (Sig1R) represents an interesting target for epilepsy management. Antiseizure effects of the positive allosteric modulator E1R have already been reported in acute seizure models. Although modulation of serotonergic neurotransmission is considered the main mechanism of action of fenfluramine, its interaction with Sig1R may be of additional relevance.

Methods: To further explore the potential of Sig1R as a target, we assessed the efficacy and tolerability of E1R and fenfluramine in two chronic mouse models, including an amygdala kindling paradigm and the intrahippocampal kainate model. The relative contribution of the interaction with Sig1R was analyzed using combination experiments with the Sig1R antagonist NE-100.

Results: Whereas E1R exerted pronounced dose-dependent antiseizure effects at well-tolerated doses in fully kindled mice, only limited effects were observed in response to fenfluramine, without a clear dose dependency. In the intrahippocampal kainate model, E1R failed to influence electrographic seizure activity. In contrast, fenfluramine significantly reduced the frequency of electrographic seizure events and their cumulative duration. Pretreatment with NE-100 reduced the effects of E1R and fenfluramine in the kindling model. Surprisingly, pre-exposure to NE-100 in the intrahippocampal kainate model rather enhanced and prolonged fenfluramine's antiseizure effects.

Significance: In conclusion, the kindling data further support Sig1R as an interesting target for novel antiseizure medications. However, it is necessary to further explore the preclinical profile of E1R in chronic epilepsy models with spontaneous seizures. Despite the rather limited effects in the kindling paradigm, the findings from the intrahippocampal kainate model suggest

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that it is of interest to further assess a possible broad-spectrum potential of fenfluramine.

KEYWORDS

amygdala kindling model, antiseizure medication, E1R, fenfluramine, intrahippocampal kainate model, NE-100

1 | INTRODUCTION

Antiseizure medications (ASMs) are the first-line therapeutic option for the treatment of epilepsies. Although many different ASMs are available, seizure control cannot be achieved in up to 30% of patients.¹ Moreover, most of the available ASMs have no impact on the pathophysiological mechanisms of epilepsies.²

Preclinically, rational polytherapy with different ASMs targeting multiple relevant mechanisms of action can exert disease-modifying effects.^{3,4} Another approach is to modulate only a single target that interacts with multiple homeostatic pathways. The sigma-1 receptor (Sig1R) could represent such a target structure. Sig1R is a chaperone protein that is activated by cellular stress, modulates various pathological processes, and maintains or restores cellular homeostasis.^{5,6} Findings from animal models of different neurological disorders provide evidence that positive pharmacological modulation of Sig1R function has beneficial, neuroprotective effects.^{7–10}

So far, little is known about the effects of Sig1R modulation in epileptology. Available data are mostly limited to findings from acute seizure models.^{11–13} Whereas Sig1R agonists have no relevant effects on acute seizures in these models, methyl phenylpiracetam (E1R), a newly designed positive allosteric modulator of Sig1R, exerts relevant antiseizure effects.¹³ These effects are completely blocked when E1R is coadministered with NE-100, a Sig1R antagonist that shows proconvulsant effects at higher doses.¹³ However, acute seizure models hardly reflect the complexity of the epileptic brain, and their predictive validity is limited.^{14,15} In addition to its antiseizure properties, Sig1R targeting may also mediate disease-modifying effects by interacting with relevant targets involved in the pathophysiological mechanisms of epilepsies.^{16–19}

Interestingly, a Sig1R-modulating effect has been reported for fenfluramine, a drug recently licensed as an add-on treatment option for patients with Dravet and Lennox–Gastaut syndrome.^{20–22} Although a positive modulation of serotonergic neurotransmission appears to be the main mechanism for fenfluramine's antiseizure effects, an additional modulation of Sig1R is discussed.^{19,22–24} However, the relative contribution of fenfluramine's

Key points

- E1R exerts dose-dependent antiseizure effects in a mouse amygdala kindling model.
- Fenfluramine exerts antiseizure effects in the mouse intrahippocampal kainate model.
- Sigma-1 receptor is confirmed as an interesting target for epilepsy management.
- The preclinical data suggest that fenfluramine may have a broad-spectrum potential in epilepsy.

interaction with Sig1R to its antiseizure effects remains unknown.

In this study, we evaluated the antiseizure effects of pharmacological Sig1R modulation in two chronic epilepsy mouse models of temporal lobe epilepsy. Moreover, we aimed to determine the relative contribution of the Sig1R interaction to the effects of fenfluramine in the same mouse models.

2 | MATERIALS AND METHODS

2.1 | Animals

Female HsdWin:NMRI mice ($n=46$, 24–32 g, 10 weeks old, Inotiv [formerly Envigo]) were used for the amygdala kindling model, and male C57BL/6J mice ($n=18$, 20–25 g, 8 weeks old, Janvier) were used for the intrahippocampal kainate (IHK) model. Male C57BL/6J mice ($n=20$, 24–33 g, 10–21 weeks old, Janvier) and female HsdWin:NMRI mice ($n=20$, 27–38 g, 17–20 weeks old, Inotiv [formerly Envigo]) were used for the analysis of serum and brain concentrations of fenfluramine and norfenfluramine. All animals were housed under standardized conditions ($22 \pm 2^\circ\text{C}$, $55 \pm 10\%$ humidity, and 12-h dark–light cycle) with ad libitum access to food and tap water (detailed information about the animal welfare principles can be found in the [Supplementary Information](#)). All experiments were conducted in line with the German Animal Welfare Act and EU Directive 2010/63/EU. Data

are reported according to the Animal Research: Reporting of In Vivo Experiments guidelines 2.0 and the Basel declaration. All studies were approved by the government of Upper Bavaria (license number ROB-55.2-2532.Vet_02-20-99). A list of the compounds used in these experiments can be found in the [Supplementary Information](#).

2.2 | Amygdala kindling model

2.2.1 | Surgery and electrode implantation

Following a habituation period of at least 1 week, animals underwent a craniotomy to implant a stimulation and recording electrode in the right amygdala. Animals received analgesia before and after the surgical procedure ([Supplementary Information](#)). Anesthesia was induced with 4% isoflurane (Isofluran CP, CP-Pharma Handelsgesellschaft) in 100% oxygen (Linde, Gases Division) and maintained with 2% isoflurane. A bipolar Teflon-isolated stainless-steel electrode was placed in the right amygdala (anteroposterior: -1.0 , lateral: $+3.2$, dorsoventral: -5.2 ; coordinates relative to bregma in millimeters) for the electroencephalographic (EEG) recordings and electrical stimulation. The electrode and screws (Schrauben-Preisinger, DIN 84 V2A M1,6X3) were fixed with dental acrylic cement (Paladur powder, Kulzer).

2.2.2 | Amygdala kindling

The initial afterdischarge threshold (ADT) was determined 2 weeks after surgery. Animals were then stimulated once per day from Monday to Friday with the same electrical pulse of 700 or 840 μA ([Supplementary Information](#)). Seizure parameters at threshold stimulation were documented, including seizure severity (SS; based on a modified Racine scale²⁵), motor seizure duration (SD), and electrographic afterdischarge durations 1 and 2 (ADD1 and ADD2^{26,27}; [Supplementary Information](#)). ADD2 is hereafter referred to as the total afterdischarge duration (ADDT). Animals were considered “fully kindled” if they exhibited 10 generalized seizures (Racine IV or V) in response to stimulation. Because the kindled state is often less stable in mice as compared to rats, it was not a prerequisite for a “fully kindled” state that all of these generalized seizures occurred consecutively.

The postkindling ADT was then determined. If animals did not present a generalized motor seizure at the postkindling ADT (ADT = generalized seizure threshold [GST]), stimulation was continued until generalized seizure activity was induced. This current intensity is defined as GST. As soon as the repeatedly determined ADTs did

not differ by more than $\pm 20\%$ (three-step range) and the animals reliably exhibited generalized seizure activity at the ADT determination, the ADT was considered stable, and alternating vehicle–drug experiments were initiated.

2.2.3 | Vehicle–drug experiments

Treatment of animals alternated between the vehicle (saline) and the test compounds, with at least 1 day between the vehicle and compound experiments. A washout period of at least 96 h was applied between two consecutive drug experiments. On each experimental day, ADT/GST and associated seizure parameters (SS, SD, ADD1, and ADDT) were assessed following a gradually increasing stimulation procedure with a 20% increase in current intensity with an initial current below the threshold value identified as stable. This procedure was followed to minimize the duration of the experiments.

Drugs were injected with the following pretreatment times: 80 min (NE-100), 60 min (E1R), and 30, 60, 120, and 240 min (fenfluramine). To confirm the relevance of the compound's interaction with Sig1R for the observed effects and to determine the relative contribution of the compound's interaction with Sig1R to the effects, combinations of the Sig1R ligands (E1R or fenfluramine) and the antagonist NE-100 were assessed.

Data were collected from two different animal groups (group 1: E1R, NE-100, and E1R/NE-100 in combination, and fenfluramine 10 mg/kg with a pretreatment time of 30 min; group 2: fenfluramine and fenfluramine/NE-100 in combination; [Supplementary Information](#)).

2.2.4 | Assessment of tolerability and motor side effects

Before starting the vehicle–drug experiments, the tolerability of 25, 35, and 45 mg/kg NE-100 and 45 mg/kg fenfluramine was tested in two fully kindled animals. We expected a good tolerability of E1R based on the communication with our collaborators at the Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis (LIOS), Riga, Latvia, and a respective published reference by Zvejniece and colleagues.²⁸ In this study, the effects of different doses of E1R on locomotion (open field test), coordination (rotarod and chimney test), and muscular strength (traction tests) were investigated in mice.

To analyze the effects of the test substances on motor coordination, rotarod performance was assessed for all vehicle and drug experiments at three different time points: directly before vehicle/drug injection (t_0), after half of the

pretreatment period (t_1), and directly before ADT evaluation (t_2). Animals were also carefully observed for signs of other adverse effects at these time points (Supplementary Information).

2.3 | IHK model

2.3.1 | Surgery, electrode implantation, and intrahippocampal injection of kainate

Following habituation, mice underwent surgery with a telemetric transmitter (ETA F10; Data Sciences International) and electrode implantation. Animals received analgesia before and after the surgery (Supplementary Information). Mice were anesthetized with chloral hydrate (500 mg/kg ip dissolved in saline; Merck) based on studies reporting effects of inhalation anesthesia on the induction of status epilepticus (SE) in this model.²⁹ SE was induced following the protocol by Buchecker et al.³⁰ The assessment of SE is described in the Supplementary Information.

2.3.2 | Vehicle–drug experiments

Five weeks post-SE, consistent spontaneous recurrent electrographic seizure activity was confirmed and alternating vehicle–drug experiments were conducted. Animals received either the vehicle (saline or aqua ad iniectabilia) or the test compounds alternately, with an interval of 1 or 2 days between the vehicle and drug experiments. There was a washout period of at least 96 h between two consecutive drug experiments. On each experimental day, EEG seizure activity was recorded, starting with a 2-h reference baseline recording before vehicle or drug injection and lasting at least 5 h after administration of vehicle or drug injection. The analysis of EEG data is described in the Supplementary Information.

2.4 | Tissue preparation and analysis of serum and brain concentrations of fenfluramine and norfenfluramine

Information about the analysis of drug exposure in plasma and brain is provided in the Supplementary Information.

2.5 | Statistics

Power analysis resulted in a calculated sample size of 11 mice per group (GPower 3.1, Heinrich Heine Dusseldorf University,³¹ Supplementary Information). This number

was not achieved due to animal loss during the experiments in the IHK model (see the Supplementary Information and Figure S1). The order of animals for procedures was randomized as described in the Supplementary Information. Due to the experimental design, blinding was not feasible and therefore not performed. Data were tested for normality, and the appropriate statistical hypothesis test was used (Supplementary Information). The median effective dose (ED_{50}) was calculated based on least-squares linear regression analysis in Excel version 16.0 (Microsoft Corporation). Data are presented as mean \pm SEM. Prism (GraphPad Software, v9.4.1) and R (v4.2.2)³² were used for all statistical tests and plots. A p -value $\leq .05$ was considered statistically significant. In addition to the hypothesis-testing statistical analysis, we provide the effect sizes for all experimental comparisons; these results can be found in Tables S4–S7.

3 | RESULTS

3.1 | Effects of E1R in fully kindled mice

The choice of pretreatment times and dose range for E1R was based on extensive experimental experience and previously published data.^{13,17} Intraperitoneal administration of E1R 60 min prior to ADT determination resulted in dose-dependent increases in thresholds ($p_{50} = .0056$, $p_{75} = .0016$, $p_{100} = .0007$; Figure 1A). Whereas 25 mg/kg E1R did not exert relevant effects, increases observed in response to 50, 75, and 100 mg/kg E1R reached 44%, 75%, and 116%, respectively. The increase in thresholds was associated with a reduction of seizure severity at threshold stimulation ($p_{50} = .0033$, $p_{75} = .0038$, $p_{100} = .0462$; Figure 1B). Moreover, the duration of behavioral seizure activity (50 and 75 mg/kg E1R) and the duration of afterdischarges (50 mg/kg E1R) were shortened as a consequence of exposure to E1R ($p_{50} = .0069$, $p_{75} = .0041$, and $p_{50} = .0115$, respectively; Figures 1C,D and S2A).

Based on these experimental data, we calculated the median dose increasing the ADT by 50% (ED_{50} ; equation $y = 175.45x - 246.02$; Figure 1E). The respective ED_{50} amounted to 48.66 mg/kg.

Whenever ADT was not associated with generalized seizure activity, stimulations were continued with incremental increases until a generalized seizure (score IV or V) was elicited. In response to 50, 75, and 100 mg/kg E1R, we observed significant increases in GST, reaching a maximum of 238% following the highest dose ($p_{100} = .0007$; Figure 1F). The seizure and afterdischarge duration recorded at GST remained unaffected, except for a significant reduction in seizure duration following administration of 75 mg/kg E1R ($p_{75} = .0131$, Figures 1G,H and S2B). The

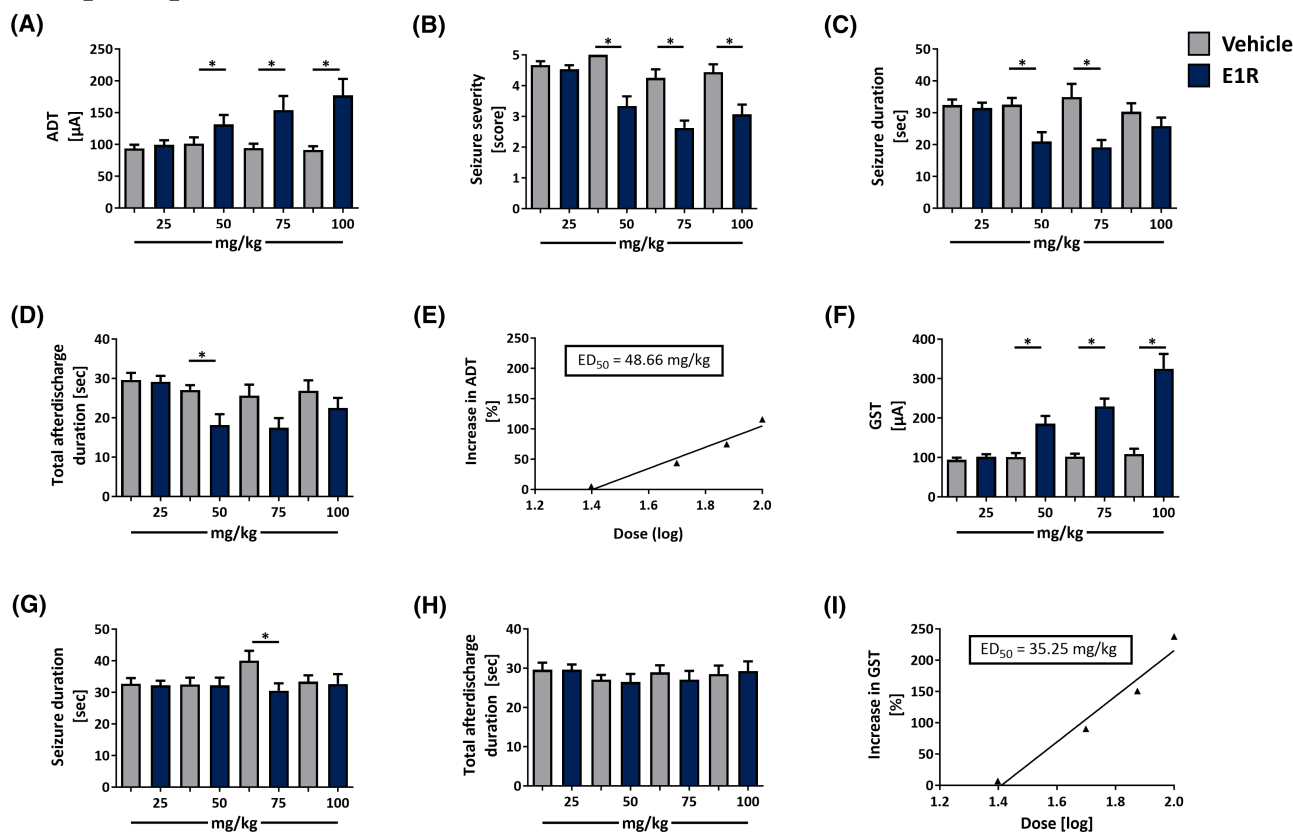


FIGURE 1 (A–D) Effect of E1R on the afterdischarge threshold (ADT) and associated seizure parameters in fully kindled mice. E1R increased the ADT in a dose-dependent manner (A). The increase in thresholds was associated with a reduction of seizure severity (B). E1R at 50 and 75 mg/kg shortened the duration of behavioral seizure activity (C) and total afterdischarge (D). The median dose that increased the ADT by 50% (ED_{50}) amounted to 48.66 mg/kg (E). (F–H) Effects of E1R on the generalized seizure threshold (GST) and associated seizure parameters in fully kindled mice. E1R increased the GST in a dose-dependent manner (F). E1R at 75 mg/kg shortened the duration of behavioral seizure activity (G) but not of the total afterdischarge duration (H), recorded at GST. The ED_{50} for GST amounted to 35.25 mg/kg (I). All data are presented as mean + SEM. * $p < .05$. Animal numbers in panels A–D and F–H were $n = 14$ –16. Statistical test used was paired Student t -test. Outliers: One data point was excluded from all doses in panels A and F. One data point was excluded from the dose 25 mg/kg in panels B–D, G, and H, respectively. Please note differences in the scaling of the y-axes in the different figures.

calculated median effective dose for a 50% increase in GST amounted to 35.25 mg/kg (equation: $y = 365.08x - 514.82$; Figure 1I).

All mice were intensely monitored following administration of E1R. All doses were well tolerated, with no evidence of relevant adverse effects. In addition, all animals successfully completed the rotarod test 30 min and 58 min after E1R injection, arguing against a relevant impact of E1R on motor coordination (Table S1).

3.2 | Effects of fenfluramine in fully kindled mice

Considering the variety of pretreatment times and times of maximum effects reported for fenfluramine in different mouse studies,¹⁹ we decided to complete a thorough efficacy and tolerability analysis evaluating different combinations of pretreatment times and doses (Table S2).

Dose-limiting adverse effects were observed in a pilot study with 30 and 45 mg/kg fenfluramine (for details see Supplementary Information). Moreover, proconvulsant effects were observed 30 min following administration of 10 mg/kg fenfluramine in kindled mice (Figures 2A–C and S3).

Based on these pilot data and the overall aim to determine a dose–response curve, we further assessed the effects of fenfluramine with a pretreatment time of 120 min and doses ranging .03–10 mg/kg (Figures 2D–F, S4, and S5, Table S2). Only minor effects were observed, with slight ADT and GST increases in response to .1 and 1 mg/kg ($p_{.1} = .0481$, $p_{.1} = .00039$ in Figure 2D; $p_{.1} = .0244$, $p_{.1} = .0265$ in Figure S3D). Moreover, a slight increase in GST was evident 120 min following administration of 10 mg/kg ($p_{10} = .0432$; Figure S3D). An influence on seizure parameters at threshold stimulation was only observed following .03 mg/kg fenfluramine, with a longer duration of behavioral seizure activity ($p_{.03} = .0268$).

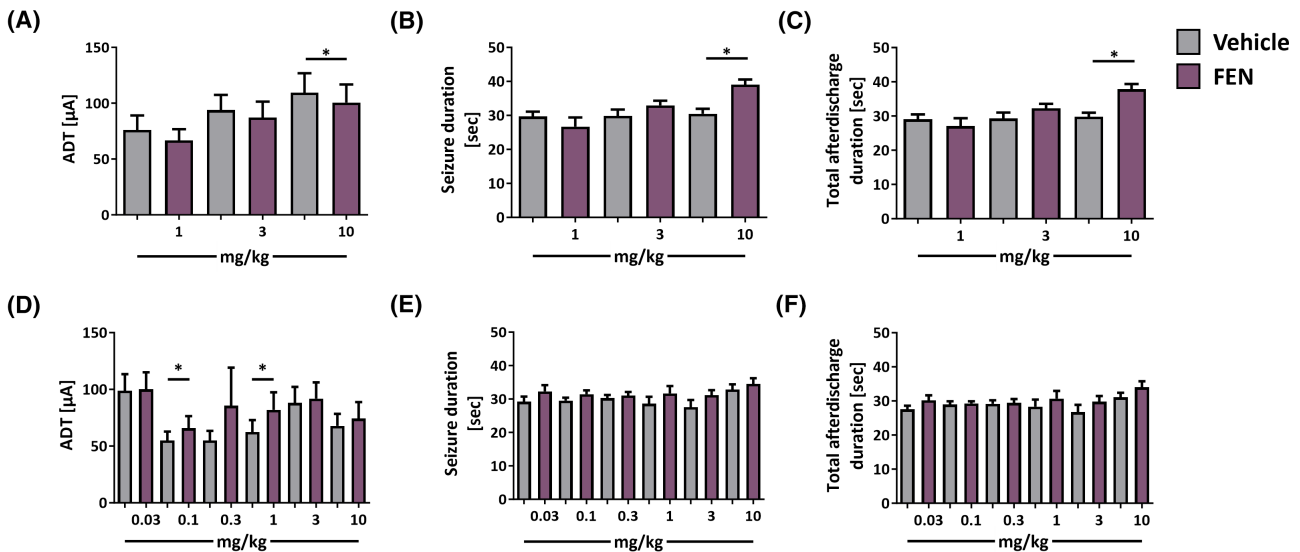


FIGURE 2 (A–C) Effects of fenfluramine (FEN) on afterdischarge threshold (ADT) and associated seizure parameters at a pretreatment time of 30 min in fully kindled mice. FEN at 10 mg/kg reduced the ADT (A) and prolonged the duration of behavioral seizure activity (B) and the total afterdischarge (C). (D–F) Effect of FEN on ADT and associated seizure parameters at a pretreatment time of 120 min in fully kindled mice. FEN at .1 and 1 mg/kg increased the ADT (D), with no effects on behavioral seizure activity (E) or the total afterdischarge duration (F). All data are presented as mean + SEM. * $p < .05$. Animal numbers in panels A–C were $n = 15$ and in D–F were $n = 12–14$. Statistical test used was paired Student t -test. Outliers: Two data points were excluded from the dose .03 mg/kg in panel D. Please note differences in the scaling of the y-axes in the different figures.

Considering doses up to 10 mg/kg, the majority of animals successfully completed the rotarod tests at both time points following fenfluramine administration (Table S1).

3.3 | Impact of sigma-1 antagonism on effects of E1R and fenfluramine in fully kindled mice

In a pilot experiment, we excluded any relevant direct effects of the sigma-1 antagonist NE-100 in fully kindled mice (Figure S6). Pretreatment with 25, 35, or 45 mg/kg NE-100 diminished the response of kindled mice to E1R, with a significant increase in GST ($p_{25} = .05$, $p_{35} = .002$, $p_{45} = .01$; Figure 3A,B).

Considering that the most prominent antagonistic effect became evident in response to 35 mg/kg NE-100, this dose was selected for subsequent combination experiments with fenfluramine. Pre-exposure to NE-100 abolished the effect of 1 mg/kg fenfluramine on ADT and GST ($p_{ADT} = .0025$, $p_{GST} = .0038$; Figure 3C,D).

3.4 | Effects of E1R and fenfluramine in the IHK model

Both doses of the positive control compound diazepam significantly reduced the frequency of high-voltage

sharp waves (HVSWs; Figure 4A) and hippocampal paroxysmal discharges (HPDs; Figure 4B), as well as cumulative and mean seizure durations (Supplemental file and Figure S7).

In apparent contrast to diazepam, E1R exposure within the range of 25–100 mg/kg did not result in any relevant effects on electrographic seizure activity (Figures 4C,D and S8).

Five different doses of fenfluramine were tested in the IHK model. Fenfluramine did not affect the frequency or cumulative duration of HVSWs or HPDs or the mean seizure duration at doses ranging from .03 to 1 mg/kg (Figures 5A,B and S9). However, fenfluramine at 10 mg/kg reduced HVSW frequency but not the cumulative duration of HVSWs during the first 2 h after its administration (Figures 5A and S9A). In contrast, there was no significant impact of fenfluramine on frequency of HPDs (Figure 5B). The total reduction of electrographic seizure activity during the first 2 h following administration reached a mean of 81% ($p = .0004$) as compared to the baseline (Figure 5C).

3.5 | Impact of sigma-1 antagonism on effects of fenfluramine in the IHK model

Administration of 35 mg/kg NE-100 remained without effects on seizure frequencies, cumulative seizure duration,

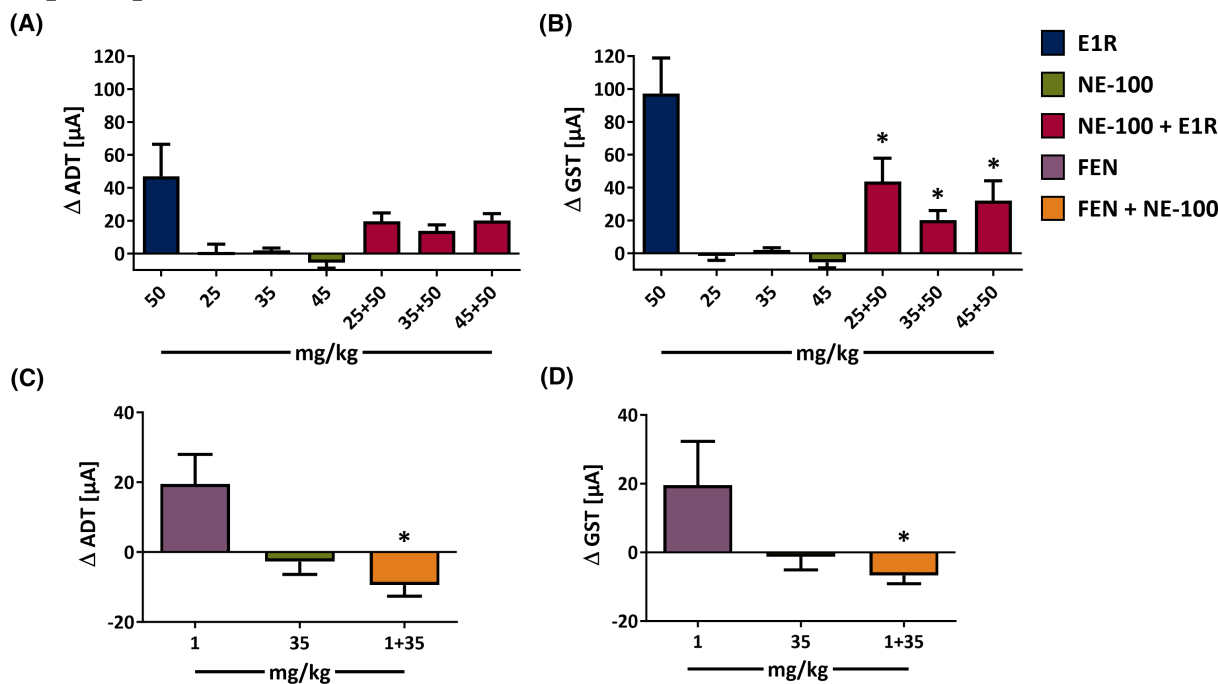


FIGURE 3 (A, B) Impact of sigma-1 receptor (Sig1R) antagonism on effects of E1R in fully kindled mice. NE-100 alone had no relevant direct effects on thresholds, seizure spread, or termination. Pretreatment with NE-100 diminished the response of kindled mice to 50 mg/kg E1R treatment on generalized seizure thresholds (B). (C, D) Impact of Sig1R antagonism on effects of fenfluramine (FEN) in fully kindled mice. Pre-exposure to NE-100 partially abolished the effect of 1 mg/kg FEN on afterdischarge thresholds (ADTs; C) and on generalized seizure thresholds (GSTs; D). The delta change represents the differences in the thresholds between vehicle and E1R/FEN treatment. All data are presented as mean + SEM. * $p < .05$ versus E1R (A and B) or versus FEN (C and D). Animal numbers in panels A and B were $n = 15$ and in panels C and D were $n = 13$ –15. Statistical tests used were one-way analysis of variance, followed by Tukey honest significant difference test (A and B, E1R vs. all NE-100+E1R) and paired Student *t*-test (C and D, FEN vs. FEN+NE-100).

or mean duration of electrographic seizures in the IHK model (Figure S10).

Surprisingly, pretreatment of animals with NE-100 20 min prior to injection of fenfluramine resulted in a more pronounced and longer lasting reduction of electrographic seizure activity (Figures 5D–F and S11). In an additional control experiment, we administered NE-100 40 min after fenfluramine (matching the pretreatment time from the kindling experiments). In this experiment, we observed no impact on fenfluramine effects except for an impact on the duration of the antiseizure effect (Figure S12). Overall, the duration of seizure reduction in the combination experiments was prolonged to 130 min after the administration of fenfluramine.

4 | DISCUSSION

The kindling paradigm is a chronic model with an excellent predictive validity and translation of preclinical findings to clinical efficacy in the management of focal onset seizures.^{14,33,34} The vast majority of approved ASMs show efficacy in electrical kindling paradigms with stimulation via depth electrodes.^{35,36} Thus, the dose-dependent effects

of E1R in this paradigm suggest a relevant antiseizure effect, with an increase in seizure thresholds reflecting an anti-ictogenic effect. In addition, the data point to a relevant influence on spread and termination of seizure activity. These findings are in line with earlier reports describing an antiseizure effect of E1R in acute models with induction of seizure activity by pentylenetetrazol (PTZ) or bicuculline.¹³ The confirmation of an antiseizure effect in a chronic electrical kindling paradigm is of particular relevance considering that acute models do not recapitulate the complex alterations in the epileptic brain, which can influence drug responsiveness. In this context, it also needs to be considered that the predictive validity of the PTZ model has been questioned considering false positives and negatives that have not translated into clinical efficacy against myoclonic and absence seizures.^{14,37} Moreover, both models are biased by the GABAergic mechanisms of the chemoconvulsant, which may result in the selection of drug candidates with a specific mechanism of action.

An assessment of drug candidates should consider efficacy in relation to tolerability.^{35,38} Although most ASMs influence kindled seizures, it is well known that relatively high doses are often required in electrical kindling

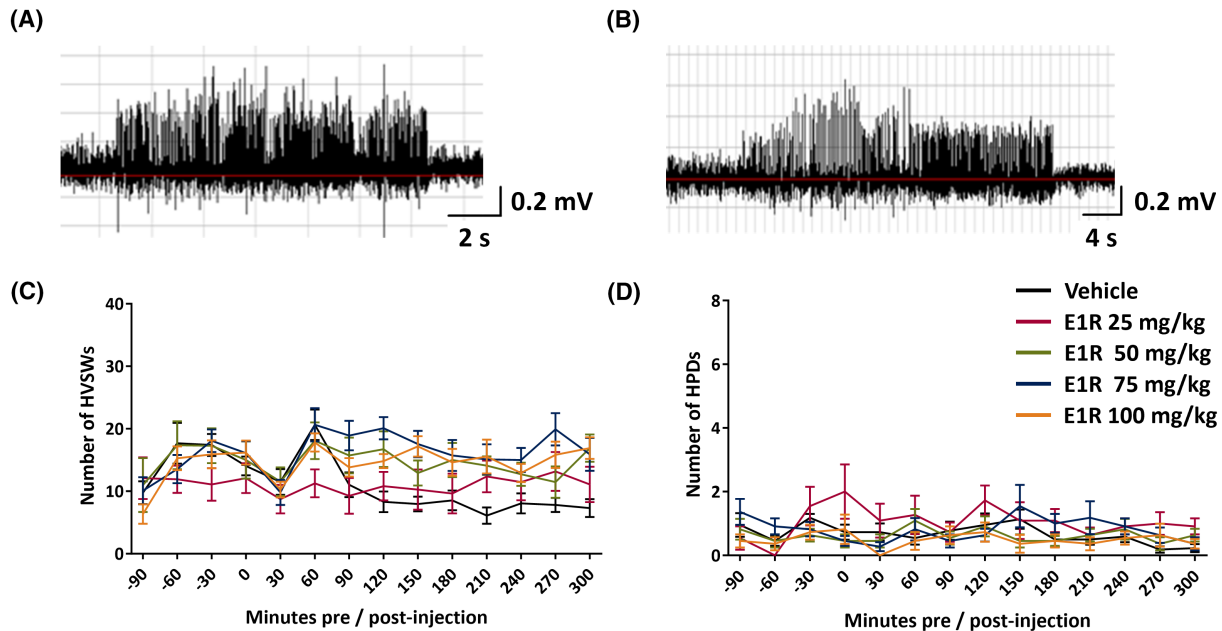


FIGURE 4 (A, B) Example illustration of high-voltage sharp waves (HVSWs; A) and hippocampal paroxysmal discharges (HPDs; B). (C, D) Effects of E1R in the intrahippocampal kainate model. Exposure to E1R in the range of 25–100 mg/kg did not result in any relevant effects on HVSWs (C) or HPDs (D). The animals were injected at time point 0. The graphs illustrate the total number of HVSWs or HPDs during 30-min time windows, with the data points summarizing the data from the preceding 30 min. All data are presented as mean \pm SEM. Animal numbers in panels C and D were $n = 11$. Statistical tests used were Friedman test followed by pairwise comparisons using the paired Wilcoxon test and Bonferroni correction.

paradigms, often approaching median toxic dose levels with motor adverse effects.³⁵ In apparent contrast, E1R exposure was well tolerated, without evidence of relevant motor adverse effects in the dose range tested.

Surprisingly, fenfluramine exerted only limited effects, with increases in ADT, reaching a maximum of 31% and lacking a clear dose dependency (Figure 2D). Based on our pilot study, we can rather exclude that the failure to show more pronounced effects is due to a suboptimal pre-treatment time. In this context, it is of interest that the concentration analysis in serum and plasma points to a T_{max} between 30 and 60 min. However, the proconvulsant effects 30 min following administration of 10 mg/kg fenfluramine argued against an intensified testing at this earlier time point. Moreover, we observed dose-limiting adverse effects at higher doses, so it was impossible to further increase the dose.

The mouse IHK model is a chronic paradigm with induction of a SE triggering epileptogenesis and manifestation of a chronic epileptic state.³⁹ The model has been suggested as a model of drug-refractory epilepsy, based on the observation that many available ASMs fail to exert efficacy at well-tolerated doses.^{14,15,39} It should be taken into account that the model seems to have some selectivity toward drugs that enhance GABAergic signaling.³⁹ In this context, it is of particular interest that fenfluramine, which does not directly affect GABAergic signaling, decreased

the number and the cumulative duration of electrographic seizure events at the highest dose tested (10 mg/kg).

In apparent contrast to fenfluramine, E1R did not exert antiseizure effects in the IHK model. Considering the broad dose range that we tested, these data strongly argue against a relevant impact of positive allosteric Sig1R modulation in this model of drug-refractory epilepsy. However, it should be taken into account that the choice of the mouse strain, the type of electroencephalographic seizure events, and the exact HVSW definition can influence pharmacoresponsiveness.⁴ In this context, it is important to note that our E1R data support an impact on neither HVSWs nor HPDs.

Comparison of E1R and fenfluramine data from the chronic models with repeated induction of focal onset seizures versus spontaneous recurrence of focal seizures provides the first evidence for a different spectrum of pre-clinical efficacy. Interestingly, the models seem to distinguish between both compounds. Earlier findings from the IHK model already demonstrated that ASMs that exert relevant antiseizure effects in a kindling paradigm can fail in this chronic model with frequent electrographic seizures.³⁹

The failure to demonstrate antiseizure effects in the IHK model might argue against Sig1R-positive allosteric modulation as a mechanism of interest for management of multidrug-refractory epilepsy. For interpretation of the efficacy in the kindling paradigm, it should be considered

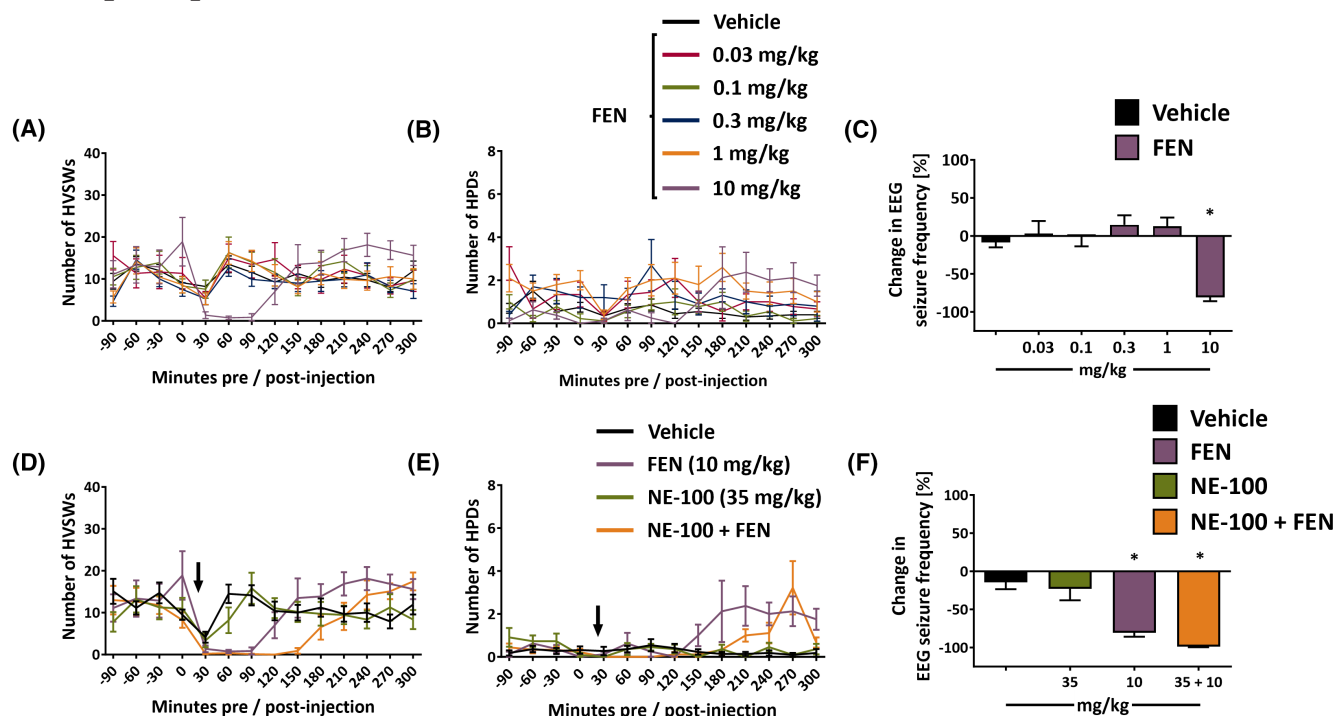


FIGURE 5 (A–C) Effects of fenfluramine (FEN) in the intrahippocampal kainate model. FEN at 10 mg/kg reduced the frequency of high-voltage sharp waves (HVSWS; A) but not of the hippocampal paroxysmal discharges (HPDs; B). This effect was evident when comparing the percentage of change in the electroencephalographic (EEG) seizure frequency (HVSWS + HPDs) between 2 h before and after the administration of the drug/vehicle (C). (D–F) Impact of sigma-1 receptor (Sig1R) antagonism on effects of FEN in the intrahippocampal kainate model. Administration of 35 mg/kg NE-100 remained without effects on HVSWS (A) or HPD (B) frequencies. Pretreatment of mice with NE-100 20 min prior to injection of FEN resulted in a longer lasting reduction in HVSWS and a reduction in the percentage of change in the EEG seizure frequency (C). The animals were injected at time point 0. The arrow indicates the time point of the second injection in the NE-100+FEN and vehicle groups (D and E). The percentage of change presented in C and F was calculated from the EEG seizure frequency (HPDs + HVSWS) of the first 2 h after drug/vehicle administration compared to the 2 h before drug/vehicle administration. All data are presented as mean \pm SEM. * $p < .05$ versus vehicle. Animal numbers in panels A–C were $n = 8–9$ and in panels D–F were $n = 8–11$. Statistical tests used were Friedman test followed by pairwise comparisons using the paired Wilcoxon test with Bonferroni correction (A, B, D, and E) and Kruskal–Wallis test followed by Dunn test (C and F). Please note that the estimated sample size was not achieved for these experiments, and the calculated effect sizes (with 95% confidence intervals) can be found in Tables S5–S7.

that the kindling paradigm has an excellent predictive validity for efficacy against focal onset seizures in patients with epilepsy, as confirmed by a retrospective comparison of clinical data with preclinical data. To our knowledge, the kindling paradigm has been the only model to accurately predict the efficacy of levetiracetam in human epilepsy. In addition, cenobamate, a recently approved ASM, also showed antiseizure effects in this paradigm, confirming the excellent predictive validity of the kindling model even with more recently approved ASMs.^{34,35}

On the other hand, it must also be considered that the different face validity of the models can have implications for the actual predictive validity, in particular concerning an efficacy in common drug-refractory epilepsies. In this context, it needs to be taken into account that we used a standard kindling approach and not one of the variants that have been suggested as tools to select drug candidates for drug-refractory epilepsy, for example, preselected ASM

nonresponders or lamotrigine-exposed kindled rats or mice.^{15,36}

In contrast to the amygdala kindling paradigm, the IHK model is characterized by frequent spontaneous EEG seizure events, thus better recapitulating the main hallmark of chronic epilepsy.⁴⁰ Moreover, the IHK model has been suggested as a model of drug-refractory epilepsy based on the observation that many ASMs fail to prevent seizures in this paradigm at well-tolerated doses.³⁹ As mentioned above, the failure of E1R to prevent electrographic seizure events in this model may thus argue against the value of Sig1R as a target for drug-refractory epilepsies, but it should also be borne in mind that robust retrospective confirmation of the predictive validity of the IHK model for drug-refractory epilepsy is still awaited. Therefore, it may still be of interest to further investigate the efficacy pattern of E1R in follow-up studies in other chronic models that recapitulate features of drug-refractory epilepsy.

Considering that E1R is a selective positive allosteric modulator of Sig1R, the contrasting findings with E1R and fenfluramine in the chronic models already suggest that an interaction with Sig1R does not play a major role for the efficacy of fenfluramine in the IHK model. A relevant contribution of an interaction with Sig1R for fenfluramine's antiseizure effects has been initially suggested by findings in a Dravet zebrafish model.²² In a follow-up study, Martin et al.¹⁹ demonstrated that fenfluramine can positively modulate Sig1R in a binding immunoglobulin protein/Sig1R dissociation assay. The authors suggested that fenfluramine might rather potentiate the response to Sig1R agonists.¹⁹ Although the exact mechanism and mode of action for fenfluramine remains unknown, there is cumulating evidence that fenfluramine positively modulates Sig1R.^{41,42} Furthermore, there is evidence that most of the positive Sig1R modulators (including fenfluramine) may show a biphasic effect depending on the concentration.^{41,43} Taken together, different mechanisms and modes of action for E1R and fenfluramine must be considered when interpreting the different efficacy profiles found in these models. Thus, it might be of future interest to assess combinations of fenfluramine with a Sig1R agonist in chronic epilepsy models.

On the other hand, it might also be that the interaction with Sig1R plays a larger role in models of Dravet syndrome. This is a hypothesis that requires further testing in Dravet mouse models. Taken together, our data provide evidence that the response to fenfluramine might be predominantly related to an alternative mechanism of action, for example, the positive modulation of serotonergic neurotransmission with effects on extracellular serotonin concentrations and at different serotonergic receptors and/or dose-dependent biphasic effects on Sig1R.^{22,24,41–44} However, in this study, we have only shown that the effect of fenfluramine is not mediated via Sig1R. Therefore, further experiments with serotonergic antagonists or measurements of serotonin levels in murine brains in the IHK model are needed to clarify the involvement of the probable main mechanism of action, the positive modulation of serotonergic neurotransmission.

The combination experiments with the Sig1R antagonist NE-100 indicate that a modulation of Sig1R by fenfluramine might only play a role in the kindling paradigm. However, the data from the combination experiment require cautious interpretation, as the antiseizure effects of fenfluramine were not very pronounced in the kindling paradigm. Moreover, NE-100 is not the most potent Sig1R antagonist, and it can induce generalized seizures (75 mg/kg) and sensitize to pentylentetrazole-induced seizures (25 mg/kg), which limits its use at higher doses.¹³ Therefore, future studies with another, more potent Sig1R antagonist may be of interest to further investigate the

contribution of Sig1R to the effects of fenfluramine. On the other hand, NE-100 at lower doses (1 and 5 mg/kg) has been shown to efficaciously block the Sig1R-mediated effects of fenfluramine and other Sig1R agonists in *in vivo* models of amnesia, supporting our choice of this Sig1R antagonist.^{19,45}

Considering cellular expression levels of Sig1R, it should be considered that the different effects in the tested models may also be related to a differential expression level of Sig1R in brain regions with neuronal cell stress and damage. To our knowledge, there is a lack of respective information on differential expression patterns in the hippocampus of animals during the chronic phase of the IHK model or in the amygdala of kindled mice. In a very comprehensive and elaborate study, including unbiased stereological cell counting, we are currently investigating the expression patterns of Sig1R in different brain regions involved in epileptogenesis and epilepsy in both models. The knowledge gained from this study could provide another explanation for the different activity of these compounds.

That modulators of GABAergic neurotransmission tend to have a better efficacy in the IHK model³⁹ would be in line with a predominant relevance of the impact of fenfluramine on serotonergic neurotransmission for its effects in this paradigm. In a simplified model, it has been suggested that the impact of fenfluramine on serotonin signaling mediates an enhancement of inhibitory GABAergic signaling, whereas the interaction with Sig1R proteins may rather limit glutamatergic neurotransmission.¹⁹

Fenfluramine has been approved as an adjunctive therapy for two developmental and epileptic encephalopathies, Dravet and Lennox–Gastaut syndromes. Considering the interest in expanding indications from rare indications with orphan drug designation to a broad application in common drug-refractory epilepsy with different etiologies, it is of particular relevance to check available preclinical data for evidence of a broad-spectrum potential. Preclinical data for fenfluramine tend to be difficult to interpret because of contrasting findings, and as stated above, evidence exists that T_{max} in the brain and optimal pretreatment times may vary depending on the mouse strain, further animal characteristics, and the seizure or epilepsy model used. Beyond efficacy testing in Dravet models, preclinical assessment so far comprised an analysis in different acute seizure assays. Whereas Sileniaks et al.⁴⁶ reported a lack of significant effects in the maximum electroshock seizure (MES) test, the maximum electroshock seizure threshold test, and the 6-Hz (32 and 44 mA) test, Martin et al.¹⁹ described relevant antiseizure effects in the MES and the 6-Hz 44-mA tests. Moreover, testing in a model of audiogenic seizures revealed antiseizure effects.⁴⁷ The

fact that chronic models better recapitulate epilepsy-associated alterations, and therefore are characterized by a higher target validity,¹⁵ underscores the relevance of data from kindling and post-SE models. Our findings from both models suggest that it is worthwhile to further explore a broad-spectrum potential of fenfluramine and that it might be possible to further expand the clinical indications in the long run.

In conclusion, our findings reveal a different preclinical efficacy profile of the Sig1R positive allosteric modulator E1R and the ASM fenfluramine. The kindling data further support Sig1R as an interesting target for novel ASMs. However, considering the failure in the IHK model, it is necessary to further explore the preclinical profile of E1R in chronic epilepsy models, including studies that we have planned to explore a potential disease-modifying effect.

Despite the rather limited effects in the kindling paradigm, the findings from the IHK model suggest that it may be of interest to further assess a possible broad-spectrum potential of fenfluramine.

AUTHOR CONTRIBUTIONS

Daniel Pérez-Pérez: Methodology; software; validation; formal analysis; investigation; data curation; writing—original draft; writing—review & editing; visualization. **Cristina Monío-Baca:** Methodology; validation; formal analysis; investigation; data curation; writing—original draft; writing—review & editing; visualization. **Eva-Lotta von Rüden:** Conceptualization; methodology; formal analysis; data curation; writing—original draft; writing—review & editing; visualization; supervision; project administration; funding acquisition. **Verena Buchecker:** Conceptualization; methodology; supervision; project administration. **Amelie Wagner:** Methodology; validation; formal analysis; investigation. **Katharina Schönhoff:** Methodology; validation; formal analysis; investigation; writing—original draft. **Liga Zvejniece:** Conceptualization; resources; writing—review & editing. **Dennis Klimpel:** Methodology; validation; investigation; resources. **Heidrun Potschka:** Conceptualization; methodology; resources; writing—original draft; writing—review & editing; supervision; project administration; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

H.P. has received fees for consulting and presentations from Zogenix. The remaining authors have no conflicts of interest. 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.

DATA AVAILABILITY STATEMENT

Data and processing scripts can be accessed through Figshare (DOI: 10.6084/m9.figshare.26014555).

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REFERENCES

1. Kalilani L, Sun X, Pelgrims B, Noack-Rink M, Villanueva V. The epidemiology of drug-resistant epilepsy: a systematic review and meta-analysis. *Epilepsia*. 2018;59:2179–93.
2. Löscher W, Potschka H, Sisodiya SM, Vezzani A. Drug resistance in epilepsy: clinical impact, potential mechanisms, and new innovative treatment options. *Pharmacol Rev*. 2020;72:606–38.
3. Schidlitzki A, Bascañana P, Srivastava PK, Welzel L, Twele F, Töllner K, et al. Proof-of-concept that network pharmacology is effective to modify development of acquired temporal lobe epilepsy. *Neurobiol Dis*. 2020;134:104664.
4. Welzel L, Bergin DH, Schidlitzki A, Twele F, John M, Klein P, et al. Systematic evaluation of rationally chosen multitargeted drug combinations: a combination of low doses of levetiracetam, atorvastatin and ceftriaxone exerts antiepileptogenic effects in a mouse model of acquired epilepsy. *Neurobiol Dis*. 2021;149:105227
5. Lachance V, Bélanger S-M, Hay C, Le Corvec V, Banoung V, Lapalme M, et al. Overview of sigma-1R subcellular specific biological functions and role in neuroprotection. *Int J Mol Sci*. 2023;24:1971.
6. Penke B, Fulop L, Szucs M, Frecska E. The role of Sigma-1 receptor, an intracellular chaperone in neurodegenerative diseases. *Curr Neuropharmacol*. 2017;16:97–116.
7. Dong H, Ma Y, Ren Z, Xu B, Zhang Y, Chen J, et al. Sigma-1 receptor modulates neuroinflammation after traumatic brain injury. *Cell Mol Neurobiol*. 2016;36:639–45.

8. Francardo V, Bez F, Wieloch T, Nissbrandt H, Ruscher K, Cenci MA. Pharmacological stimulation of sigma-1 receptors has neurorestorative effects in experimental parkinsonism. *Brain*. 2014;137:1998–2014.
9. Ono Y, Tanaka H, Takata M, Nagahara Y, Noda Y, Tsuruma K, et al. SA4503, a sigma-1 receptor agonist, suppresses motor neuron damage in in vitro and in vivo amyotrophic lateral sclerosis models. *Neurosci Lett*. 2014;559:174–8.
10. Peviani M, Salvaneschi E, Bontempi L, Petese A, Manzo A, Rossi D, et al. Neuroprotective effects of the Sigma-1 receptor (S1R) agonist PRE-084, in a mouse model of motor neuron disease not linked to SOD1 mutation. *Neurobiol Dis*. 2014;62:218–32.
11. Emamghoreishi M, Shahpari M, Keshavarz M. Interaction of sigma-1 receptor modulators with seizure development in pentylenetetrazole-induced kindled mice. *Epilepsy Res*. 2019;154:74–6.
12. Guo L, Chen Y, Zhao R, Wang G, Friedman E, Zhang A, et al. Allosteric modulation of sigma-1 receptors elicits anti-seizure activities. *Br J Pharmacol*. 2015;172:4052–65.
13. Vavers E, Svalbe B, Lauberte L, Stonans I, Misane I, Dambrova M, et al. The activity of selective sigma-1 receptor ligands in seizure models in vivo. *Behav Brain Res*. 2017;328:13–8.
14. Löscher W. Animal models of seizures and epilepsy: past, present, and future role for the discovery of Antiseizure drugs. *Neurochem Res*. 2017;42:1873–88.
15. Kehne JH, Klein BD, Raeissi S, Sharma S. The National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program (ETSP). *Neurochem Res*. 2017;42:1894–903.
16. Kourrich S. Sigma-1 receptor and neuronal excitability. *Handb Exp Pharmacol*. 2017;244:109–30.
17. Vavers E, Zvejniece B, Stelfa G, Svalbe B, Vilks K, Kupats E, et al. Genetic inactivation of the sigma-1 chaperone protein results in decreased expression of the R2 subunit of the GABA-B receptor and increased susceptibility to seizures. *Neurobiol Dis*. 2021;150:105244.
18. Vavers E, Zvejniece L, Dambrova M. Sigma-1 receptor and seizures. *Pharmacol Res*. 2023;191:106771.
19. Martin P, de Witte PAM, Maurice T, Gammaitoni A, Farfel G, Galer B. Fenfluramine acts as a positive modulator of sigma-1 receptors. *Epilepsy Behav*. 2020;105:106989.
20. Knupp KG, Scheffer IE, Ceulemans B, Sullivan JE, Nickels KC, Lagae L, et al. Efficacy and safety of fenfluramine for the treatment of seizures associated with Lennox-Gastaut Syndrome. *JAMA Neurol*. 2022;79:554.
21. Lagae L, Sullivan J, Knupp K, Laux L, Polster T, Nikanorova M, et al. Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2019;394:2243–54.
22. Sourbron J, Smolders I, de Witte P, Lagae L. Pharmacological analysis of the anti-epileptic mechanisms of fenfluramine in scn1a mutant zebrafish. *Front Pharmacol*. 2017;8:191.
23. Rodríguez-Muñoz M, Sánchez-Blázquez P, Garzón J. Fenfluramine diminishes NMDA receptor-mediated seizures via its mixed activity at serotonin 5HT2A and type 1 sigma receptors. *Oncotarget*. 2018;9:23373–89.
24. Sourbron J, Lagae L. Serotonin receptors in epilepsy: novel treatment targets? *Epilepsia Open*. 2022;7:231–46.
25. Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972;32:281–94.
26. Boldt L, Koska I, Maarten van Dijk R, Talbot SR, Miljanovic N, Palme R, et al. Toward evidence-based severity assessment in mouse models with repeated seizures: I. Electrical kindling. *Epilepsy Behav*. 2021;115: 107689
27. Von Rüden EL, Wolf F, Gualtieri F, Keck M, Hunt CR, Pandita TK, et al. Genetic and pharmacological targeting of heat shock protein 70 in the mouse amygdala-kindling model. *ACS Chem Neurosci*. 2019;10:1434–44.
28. Zvejniece L, Vavers E, Svalbe B, Vilskersts R, Domracheva I, Vorona M, et al. The cognition-enhancing activity of E1R, a novel positive allosteric modulator of sigma-1 receptors. *Br J Pharmacol*. 2014;171:761–71.
29. Twele F, Töllner K, Bankstahl M, Löscher W. The effects of carbamazepine in the intrahippocampal kainate model of temporal lobe epilepsy depend on seizure definition and mouse strain. *Epilepsia Open*. 2016;1:45–60.
30. Buchecker V, Koska I, Pace C, Talbot SR, Palme R, Bleich A, et al. Toward evidence-based severity assessment in mouse models with repeated seizures: (II.) Impact of surgery and intrahippocampal kainate. *Eur Surg Res*. 2023;64:89–107.
31. Faul F, Erdfelder E, Lang AG, Buchner A. G*power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39:175–91.
32. Team RC. R: a language and environment for statistical computing. 4.2.2 ed. Vienna, Austria: R Foundation for Statistical Computing; 2023.
33. Löscher W. Animal models of drug-resistant epilepsy. *Novartis Found Symp*. 2002;243:149–59; discussion 159–166, 180–185.
34. Barker-Haliski M, Steve White H. Validated animal models for antiseizure drug (ASD) discovery: advantages and potential pitfalls in ASD screening. *Neuropharmacology*. 2020;167:107750.
35. Guignet M, Campbell A, White HS. Cenobamate (XCOPRI): can preclinical and clinical evidence provide insight into its mechanism of action? *Epilepsia*. 2020;61:2329–39.
36. Löscher W, White HS. Animal models of drug-resistant epilepsy as tools for deciphering the cellular and molecular mechanisms of pharmacoresistance and discovering more effective treatments. *Cells*. 2023;12:1233
37. Löscher W, Luna-Tortos C, Romermann K, Fedrowitz M. Do ATP-binding cassette transporters cause pharmacoresistance in epilepsy? Problems and approaches in determining which antiepileptic drugs are affected. *Curr Pharm Des*. 2011;17:2808–28.
38. Löscher W, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. IV. Protective indices. *Epilepsy Res*. 1991;9:1–10.
39. Duvéau V, Pouyatos B, Bressand K, Bouyssieres C, Chabrol T, Roche Y, et al. Differential effects of antiepileptic drugs on focal seizures in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy. *CNS Neurosci Ther*. 2016;22:497–506.
40. Rusina E, Bernard C, Williamson A. The kainic acid models of temporal lobe epilepsy. *eNeuro*. 2021;8:1–24.
41. Sourbron J, Lagae L. Fenfluramine: a plethora of mechanisms? *Front Pharmacol*. 2023;14:1192022.

42. Vavers E, Zvejniece L, Maurice T, Dambrova M. Allosteric modulators of Sigma-1 receptor: a review. *Front Pharmacol.* 2019;10:223.
43. Maurice T. Bi-phasic dose response in the preclinical and clinical developments of sigma-1 receptor ligands for the treatment of neurodegenerative disorders. *Expert Opin Drug Discov.* 2021;16:373–89.
44. Clineschmidt BV, Zacchei AG, Totaro JA, Pflueger AB, McGuffin JC, Wishousky TI. Fenfluramine and brain serotonin. *Ann N Y Acad Sci.* 1978;305:222–41.
45. Martin P, Maurice T, Gammaitoni A, Farfel G, Boyd B, Galer B. Fenfluramine modulates the anti-amnesic effects induced by sigma-1 receptor agonists and neuro(active)steroids in vivo. *Epilepsy Behav.* 2022;127:108526.
46. Silenieks LB, Carroll NK, Van Niekerk A, Van Niekerk E, Taylor C, Upton N, et al. Evaluation of selective 5-HT(2C) agonists in acute seizure models. *ACS Chem Neurosci.* 2019;10:3284–95.
47. Tupal S, Faingold CL. Fenfluramine, a serotonin-releasing drug, prevents seizure-induced respiratory arrest and is anti-convulsant in the DBA/1 mouse model of SUDEP. *Epilepsia.* 2019;60:485–94.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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