

## RESEARCH ARTICLE

# Preclinical evaluation of drug treatment options for sleep-related epileptiform spiking in Alzheimer's disease

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**Abstract**

**Introduction:** There are no published data on prospective clinical studies on drug treatment options for sleep-related epileptiform spiking in Alzheimer's disease (AD).

**Methods:** Using video-EEG with hippocampal electrodes in 17 APP/PS1 transgenic male mice we assessed the effects of donepezil and memantine, anti-seizure drugs levetiracetam and lamotrigine, gamma-secretase inhibitor semagacestat, anti-inflammatory minocycline and adenosine receptor antagonist istradefylline on density of cortical and hippocampal spikes during sleep.

**Results:** Levetiracetam decreased the density of hippocampal giant spikes and cortical spikes. Lamotrigine reduced cortical single spikes and spike-wave discharges but dramatically increased hippocampal giant spikes. Memantine increased cortical single spikes and spike-wave discharges dose-dependently. Memantine and istradefylline decreased total sleep time while levetiracetam increased it. Lamotrigine decreased REM sleep duration. Other drugs had no significant effects.

**Discussion:** Levetiracetam appears promising for treating sleep-related epileptiform spiking in AD while lamotrigine should be used with caution. Donepezil at low doses appeared neutral but the memantine effects warrant further studies.

**KEYWORDS**

EEG, epilepsy, hippocampus, sleep, transgenic mouse

## 1 | BACKGROUND

Alzheimer's disease (AD) has been for long associated with increased risk of epileptic seizures.<sup>1,2</sup> However, recent studies suggest that epileptic seizures are only a culmination of a larger phenomenon, neuronal hyperexcitability,<sup>3</sup> which is not only responsible for the visible convulsive seizures but also results in subclinical epileptiform activity in brain. In three recent overnight video-EEG studies, the occurrence of epileptiform spiking in early-stage AD patients with no history of

epilepsy has ranged from 22% to 54%.<sup>4-6</sup> In all these studies, epileptiform spiking occurred without motor manifestations, most often in the temporal cortex and predominantly during non-REM (NREM) sleep. The occurrence of the epileptiform activity during sleep explains why this phenomenon has gone unnoticed over 30 years during routine daytime EEG examinations. Further, the reported occurrence may be an underestimate, since invasive foramen ovale recordings in AD patients have demonstrated focal medial temporal seizures and interictal spiking that remains invisible on scalp-EEG.<sup>7</sup>

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Sleep-related non-convulsive epileptiform spiking sounds benign but may have serious consequences in AD brains. A hallmark study by Vossel and co-workers<sup>5</sup> revealed 2.5-fold faster cognitive decline during a 5-year follow-up in AD patients with subclinical epileptiform activity compared to those without detectable activity, despite similar apolipoprotein E (APOE)  $\epsilon$ 4 prevalence and brain atrophy as detected in a subset of the subjects with MRI. The finding was recently replicated in a study with 50 AD patients of which the half with subclinical epileptiform activity showed 1.5-fold faster cognitive decline over 3 years compared to those without detectable activity.<sup>6</sup> These findings speak for the need to treat such subclinical epileptiform discharges. However, there are no published controlled clinical studies on drug treatments of this condition.

While waiting for data on ongoing clinical trials, we set out to investigate possible treatment options in a valid preclinical AD model. We have earlier demonstrated that over 60% of transgenic APP<sup>swE</sup>/PS1<sup>dE9</sup> mice (from here on APP/PS1 mice) but not their wild-type littermates exhibit at least one epileptic seizure during a 3-week 24/7 video-EEG recording.<sup>8</sup> More recently, we have found that APP/PS1 mice with a seizure-resistant C57Bl/6J background frequency display hippocampal giant spikes that are extremely rare in their wild-type littermates.<sup>9</sup> The giant spikes resemble subclinical epileptiform spikes in AD patients also in three other aspects: (1) they do not associate with muscle twitches; (2) they are generated in the hippocampus but project to the cortex and show up as surface negative spikes-wave complexes (Figure 1); and (3) they occur only during sleep.<sup>9</sup> They manifest during stage N2-3 NREM sleep as in humans but also during REM sleep, often in clusters. The presence of giant spikes in AD mouse models but not in humans probably stems from the strong hippocampal-cortical synchronous theta oscillation during REM that is absent in humans.<sup>9</sup> Importantly, giant (or interictal) spiking during sleep is not a characteristic of a single transgenic mouse line but a common feature of various amyloid plaque-forming APP or APP/PS1 lines.<sup>10,11</sup>

## 2 | METHODS

### 2.1 | Animals

Seventeen 3- to 5-month-old male APP/PS1 male mice<sup>12</sup> were used. The mice were backcrossed to C57BL/6J strain for 26 generations. The mice were housed in a controlled environment (temperature  $22 \pm 1^\circ\text{C}$ , humidity 50% to 60%, lights on 07:00 to 19:00) with food and water available ad libitum. All animal procedures were carried out in accordance with the EU Directive 2010/63 and approved by the Animal Experiment Board of Finland.

Under isoflurane anesthesia (4.5-2%) two cortical screw electrodes were attached to the left and right frontal bone (AP +1.7, ML +/-1.8). A bundle of three 50  $\mu\text{m}$  insulated stainless-steel wire electrodes (staggered 400  $\mu\text{m}$  between the tips) was inserted into the left hippocampus (AP -2.1, ML +1.3 from bregma, DV 1.7 from dura). Two screws on the occipital bone served as the ground, common reference, and anchors for dental acrylic cement for a miniature connector

### HIGHLIGHTS

- Hippocampal giant spikes in APP/PS1 mice model sleep-related epileptiform spikes in AD patients
- Levetiracetam decreased the number of giant spikes per sleep time in 7/7 mice
- Lamotrigine decreased REM-sleep duration
- Lamotrigine increased the number of giant spikes in 7/7 mice and induced a seizure in 2/7

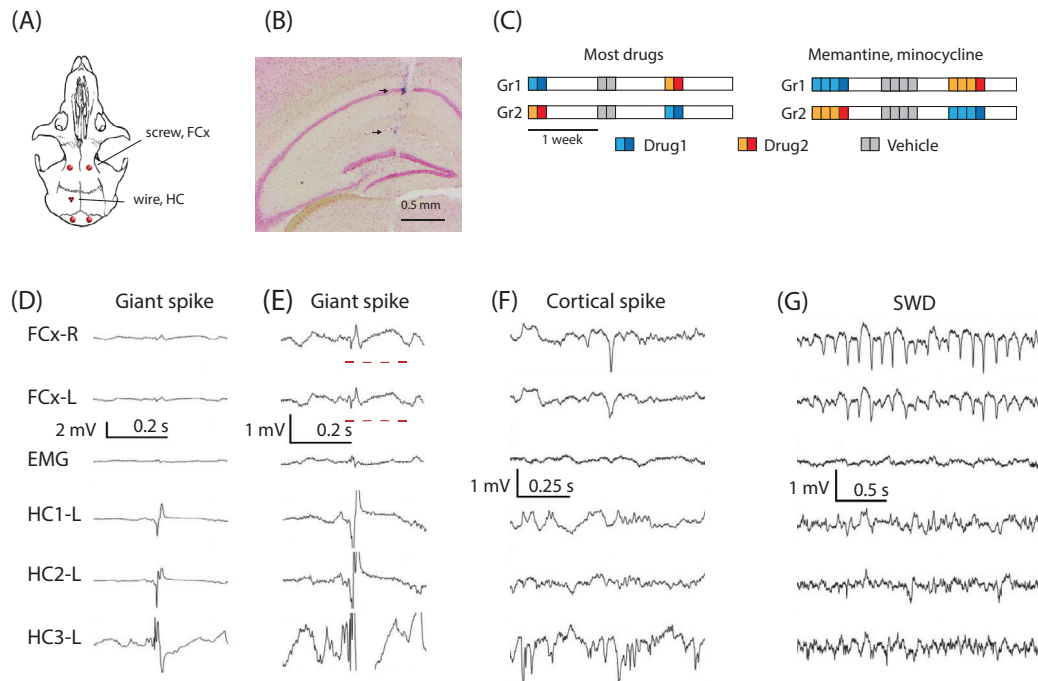
### RESEARCH IN CONTEXT

1. **Systematic review:** PubMed was used in literature reach. Recent follow-up studies link subclinical epileptiform spiking during sleep in early AD patients with significantly faster cognitive decline over 3 to 5 years. We found no clinical trials on drug treatments for the condition.
2. **Interpretation:** In a valid animal model for sleep-related epileptiform spiking in AD, levetiracetam, which reduces presynaptic glutamate release, proved effective against both hippocampal and cortical spiking. However, the glutamate NMDA-antagonist memantine did not affect hippocampal spiking and increased cortical spiking. Lamotrigine decreased cortical but dramatically increased hippocampal spiking which may remain undetected in human scalp-EEG. Donepezil as a typical anti-cholinesterase inhibitor had no significant effects on epileptiform spiking.
3. **Future directions:** Further evidence from controlled clinical trials is needed to guide drug treatment for subclinical epileptiform spiking in AD. Meanwhile, these preclinical findings speak for levetiracetam and avoidance of lamotrigine in treating epileptiform spiking in patients with early AD.

(Figure 1A). The location of the deep electrodes was confirmed histologically (Figure 1B). An additional stainless-steel wire electrode was inserted between the neck muscles for electromyogram (EMG) recording. Carprofen (5 mg/kg, i.p.) was given for postoperative analgesia.

### 2.2 | Drug administration

The tested drugs and their vehicles and doses are summarized in Table 1. They included current AD drugs, donepezil (DPZ) as a representative angiotensin-converting enzyme (ACE) inhibitor, and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine (MEM). As anti-seizure drugs we chose levetiracetam (LEV) and



**FIGURE 1** (A) Schematic illustration of the mouse skull with electrode positions. (B) Histological section stained with Prussian blue and neutral red to reveal the electrode tip positions in the hippocampus. At the end of the recording the mouse was anesthetized and small anodal current was passed through the electrode to release iron ions into the tissue which stains blue with the Prussian blue staining (arrows). (C) Administration scheme of the two drug doses with a 5-day washout in a triad and counterbalance order among the mice. (D-G) Examples of epileptiform spikes. (D) Hippocampal giant spike under vehicle injection. (E) A close-up of the same spike. Reversal of the two spike components can be seen between the second (fissure) and third (dentate granule cell layer) items. The spike component is followed by a hyperpolarization shift of ~200 ms in the hippocampal channels. Note the projection of the hippocampal discharge to the cortical channels as a spike-wave complex. (F) A cortical surface-negative spike on the right with a sharp wave of lower amplitude on the left side. Vehicle injection. (G) A spike-wave complex appears as a repeating regular pattern bilaterally in the cortical channels without any synchronous activity in the hippocampus. Memantine treatment. Abbreviations: FCx, frontal screw electrode; EMG, neck electromyogram; HC1-3, staggered hippocampal wire electrodes

**TABLE 1** Administered drugs and their dosing

Drug	Abbr.	N	Vehicle	Action	Dose mg/kg	Ref
Donepezil	DPZ	12	0.9% NaCl	ACE inhibitor	0.3-1.0 i.p.	32
Memantine	MEM	9	0.9% NaCl	NMDA antagonist	3.0-10 i.p.	33
Istradefylline	IST	12	DMSO 10%	A <sub>2A</sub> antagonist	0.3-1.0 i.p.	34
Minocycline	MIN	9	0.9% NaCl	Anti-inflammation	20-60 i.p.	13,35
Semagacestat	SGS	10	0.5% MC*	$\gamma$ -secretase inhibitor	30 i.g.	15
Levetiracetam	LEV	12	dH <sub>2</sub> O	Anti-seizure	30-100 i.p.	36
Lamotrigine	LTG	8	dH <sub>2</sub> O	Anti-seizure	15-45 i.p.	37,38

\*MC = Methyl cellulose. SGS was given as suspension via intragastric gavage due to its poor solubility 2 hours before the EEG recording.

lamotrigine (LTG), which are most often recommended for AD patients with epilepsy.<sup>3</sup> In addition, we tested three experimental drugs for mechanistic purpose: minocycline (MIN), an anti-inflammatory antibiotic that has proven anti-convulsive in several seizure models<sup>13</sup>; (2) semagacestat (SGS), a gamma-secretase inhibitor that failed to improve memory in a Phase 3 clinical trial<sup>14</sup> but proved effective in reducing amyloid- $\beta$  (A $\beta$ ) production in both mouse models<sup>15</sup> and patients; and (3) istradefylline (IST), an adenosine A<sub>2A</sub> antagonist,

based on its overexpression in AD patient and APP/PS1 mouse hippocampus and its coupling to NMDA-mediated overexcitation.<sup>16</sup> We chose the doses based on the literature so that they should not cause motor side effects.

The drugs were administered intraperitoneally at a volume of 0.1 mL/10 g 30 to 60 minutes before the video-EEG recording, with the exception of SGS and its vehicle that were given via an intragastric gavage 2 hours before due to its poor solubility. To address the

dose-response relationship, each drug (except SGS) was given at the basal dose on day 1 (low, L) and in a 3-fold dose (high, H) on day 2. The washout between the treatments was at least 5 days. The study had a within-subject design, that is, the mouse was its own control on the vehicle administration days. The drugs were administered in triads so that two study drugs were combined with their common vehicle. The order of administration was counterbalanced (A-B-C for half of mice, C-B-A for the other half) to eliminate the potential drift in baseline spiking over time (Figure 1C). MEM and MIN, as well as their common vehicle, were additionally administered at the small dose for 2 days before the video-EEG under the drugs began (Figure 1C). For MEM this was done to attain tolerance for the acute motor side effects while for MIN this was required for the full anti-inflammatory effect. The drug solutions were code-labeled and the experimenter was blind to the treatment.

### 2.3 | Video-EEG acquisition

After a week of recovery from the surgery, the mice were first familiarized with the recording environment for 2 days. The video-EEG recording took place in a circular frame made of brown compressed paper (diameter 18.5 cm, wall height 18 cm) on a translucent glass plate that was illuminated from below. Two mice in two separate frames were recorded simultaneously. One end of the recording wire was attached to a preamplifier (Plexon) that connected to the imbedded connector on the mouse's head, while the other end of the recording wire was connected to an AC amplifier (A-M Systems; gain 1000, analogue band-pass 1 to 3000 Hz). The amplified signal was digitized at 2 kHz per channel. The movements of the animals were recorded with an overhead video camera. Synchronized EEG and video signals were acquired with SciWorks 5.0 program (DataWave Technologies). Each mouse underwent one recording session of 3 hours per drug either in the morning or afternoon, but always within light phase of the day (lights on 7:00 to 19:00). Before actual drug testing the mice had two baseline sessions with a saline injection to adjust them to the injection and the recording cable.

### 2.4 | Data analysis

First, the mouse center of mass coordinates were extracted offline from the videos with commercial Ethovision software (Noldus Information Technology), and the trajectories were smoothed and corrected with customized Matlab programs, as previously reported.<sup>17</sup> Next, we assigned the mouse behavior into four states (moving, waking immobility, REM sleep and NREM sleep) with the following steps.

**Moving versus immobility.** The mouse was considered moving if its instant speed was  $> 1.2$  cm/s, otherwise immobile. To compensate insensitivity of the video detection for vertical movement, we band-pass filtered the EMG at 1 to 100 Hz and calculated its envelope. Immobility epochs with the envelope  $> 10^{12}$   $\mu$ V were assigned to the moving

state. Further, if the mouse moved  $< 0.5$  s between two brief immobility epochs or  $< 1$  s between two immobility epochs of  $> 5$  s, this short movement was assigned to immobility.

**Waking immobility versus sleep.** When immobility lasted  $> 20$  s, the first 20 s were defined as waking immobility, the rest as sleep.

**REM versus NREM.** We searched for REM in sleep epochs with low delta (1 to 4 Hz) and high theta (6 to 9 Hz) amplitudes in the top hippocampal channel. First, we searched for epochs where a delta or theta envelope was  $> 100$  mV for  $> 400$  ms and connected two epochs if the gap in between was  $< 1$  s. Epochs with only a theta envelope above these thresholds was defined as REM. If the gap between two REM epochs was  $< 10$  s, they were connected. The rest of sleep time was defined as NREM. See the Supplementary video for an example of sleep staging.

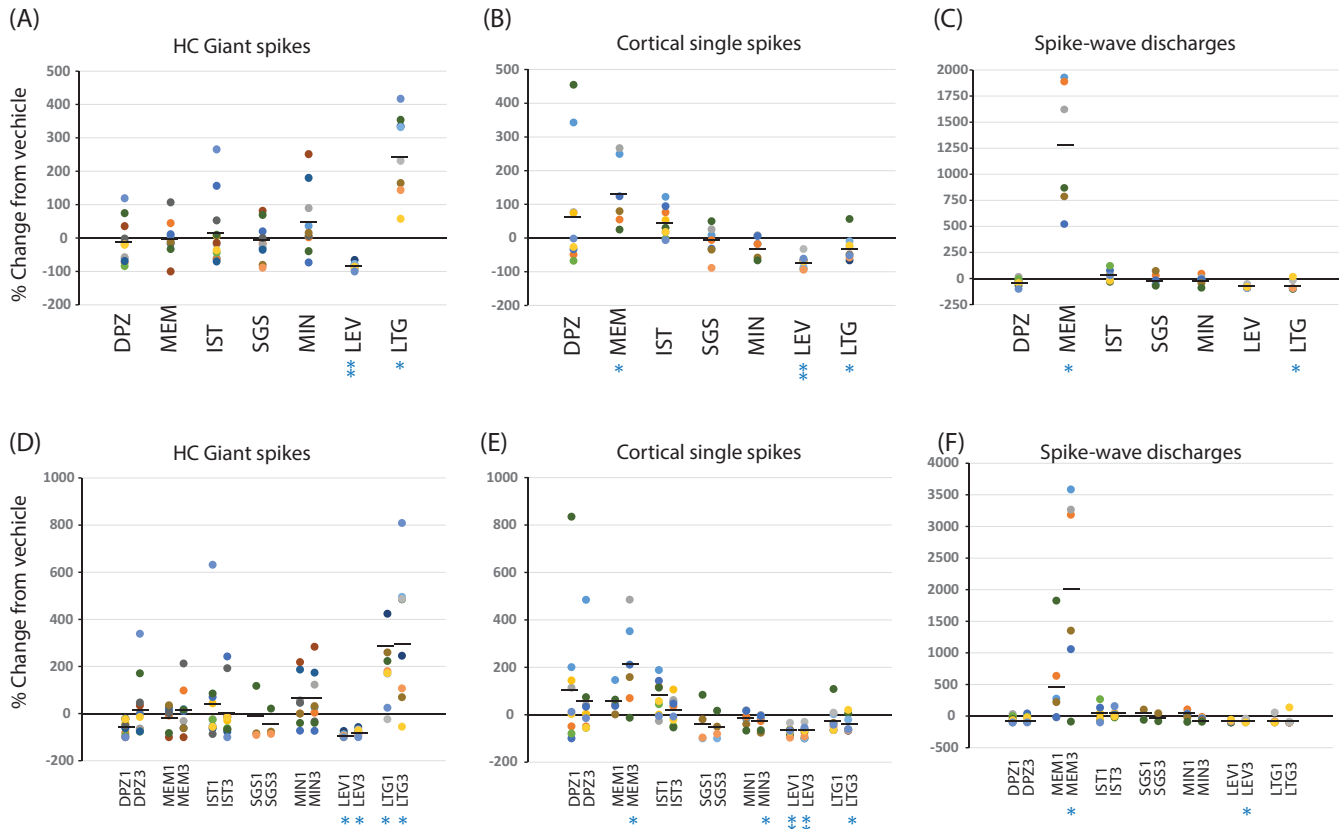
The spike extraction from the EEG was done with a customized Matlab program. First, after 50 Hz notch filtering, all channels were high-pass filtered at 8 Hz to remove slow fluctuations from the baseline. Then, the hippocampal channel with the highest deviation from baseline was selected and peaks  $> 10$  SD from the baseline and  $< 30$  ms in duration were extracted. The hippocampal spikes were considered *giant spikes* if the sharp peak was seen in all channels (one cortical channel  $> 3$  SD) and was followed by an afterhyperpolarization  $> 200$  ms in duration without concomitant EMG activity (Figure 1D,E). Next, in both frontal cortical screw channels all surface-negative peaks  $> 6$  SD below the average filtered baseline and  $< 30$  ms in duration were extracted. If the right and left spike fell within 100 ms they were considered a single event. The cortical spikes were classified as *single spikes* (Figure 1F) if they were present as singles or duplets. Trains of spikes riding on 8 to 14 Hz sleep spindles were not considered. Spikes that belong to a train of surface negative spikes and positive waves (at least 3 cycles) were treated as part of a *spike-and-wave discharge* (SWD) complex (Figure 1G).

Since epileptiform spiking in APP/PS1 mice or AD patients mostly happens during sleep, the detected spike counts were normalized to the total sleep time and expressed as spikes/h. In addition, the hippocampal giant spikes occurring during NREM versus REM sleep were easy to distinguish by the presence or absence of background theta during a sleep bout. Thus, we also calculated the density of NREM-associated giant spikes per total NREM sleep time as well as REM-associated giant spikes per total REM sleep time. The drug effects were assessed first by taking the average of spike density under the low and high dose of the active drug and calculating the difference from the corresponding two vehicle recordings of the triad:

$$\%change = (drug\ LH - vehicle\ VV) * 100 / vehicle\ VV,$$

where LH is the mean of the two drug doses and V is the vehicle given on 2 days.

The statistical testing was done with paired *t* tests between the averaged LH doses and the averaged VV administrations. To test the dose-response relationship, the paired *t* test was run between a single drug dose (L or H) and the averaged VV administrations.



**FIGURE 2** Effect of the selected drug treatments on epileptiform spiking (spikes/h of sleep) in individual mice as a relative change (%) from the corresponding vehicle treatment. (A-C) Average spiking frequency on drug (lower and higher dose). (D-F) Dose-response effect, the lower and higher doses separately. (A,D) Hippocampal giant spikes. (B,E) cortical single spikes. (C,F) cortico-thalamic spike-wave discharges. Individual mice are indicated by different colors. Short transverse lines indicate group means. Asterisks indicate a significant change from vehicle, \* $P < 0.05$ , \*\* $P < 0.01$ . Abbreviations: DPZ, donepezil; IST, istradepylline; LEV, levetiracetam; LTG, lamotrigine; MEM, memantine; MIN, minocycline; SGS, semagacestat

### 3 | RESULTS

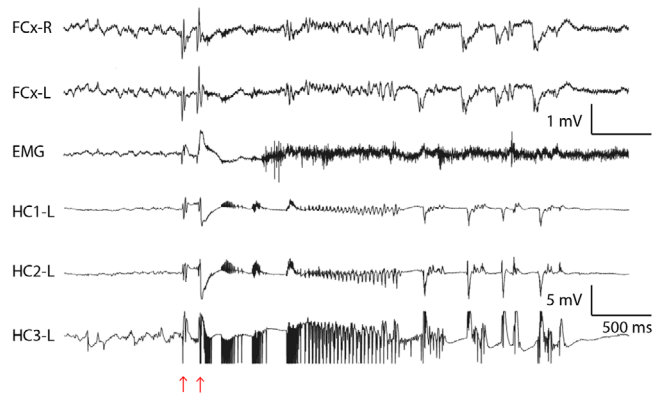
#### 3.1 | Hippocampal giant spikes

Since the number of hippocampal giant spikes varied greatly between individual animals (range 0.2 to 203.5/h during vehicle recordings), as is typical for epilepsy, all treatment effects were evaluated as percentage of change from the vehicle treatment in the same triad (Figure 1C). The treatment effects are summarized in Figure 2A. The absolute giant spike numbers per total sleep time, and per NREM and REM sleep time, are shown in supplemental Figure S1. The most consistent and statistically significant effects on hippocampal giant spikes were seen with the anti-seizure drugs. LEV decreased the number of giant spikes per hour of total sleep ( $P = .005$ ,  $n = 7$ ) and spikes occurring during NREM sleep per hour of NREM sleep ( $P = .02$ ,  $n = 7$ ). By contrast, LTG increased the density of giant spikes (per total sleep,  $P = .014$ ; per NREM sleep,  $P = .02$ ,  $n = 7$ ). Both effects were dose-independent (Figure 2D). Examples of giant spikes on vehicle, LEV, and LTG are shown in Figure S2. LEV tended to decrease the spike amplitude while LTG tended to increase it without altering the waveform.

Enhanced excitability on LTG was also seen as double giant spikes never observed under vehicle (Figure 3) and as a seizure in two mice (Figure 3), while no seizures were observed under any other treatment. In one mouse, the seizure occurred during sleep with no behavioral manifestation, whereas the other mouse moved slowly backwards like a crayfish during its two seizures. The other drugs had no significant impact on giant spikes. To further assess the dose-response dependency of LTG, we injected LTG at 15 and 75 mg/kg to two wild-type male mice of the same C57Bl/6J background with a week of washout between the injections. We seldom detect any giant spikes in C57Bl/6J wild-type mice. Unexpectedly, however, LTG induced hippocampal giant spikes during NREM sleep in both at the lower dose (12.4 vs 7.7 spikes/h of NREM sleep) but only one giant spike (0.3 spikes/h) in one mouse and none in the second mouse with the higher dose. No spiking was observed after a vehicle injection.

#### 3.2 | Cortical spiking

As a comparison to hippocampal giant spikes, we also assessed the drug effects on cortical single spikes although those are present also



**FIGURE 3** Example of a seizure induced by 15 mg/kg of lamotrigine in an APP/PS1 mouse. A double giant spike (red arrows) is followed by fast high-voltage spiking in dentate gyrus, spreads to the CA1 layer and finally to the cortex bilaterally. Note the increased tonus of the neck muscles on the EMG channel. The seizure occurred during sleep and ended upon waking up. Abbreviations: FCx, frontal screw electrode; EMG, neck electromyogram; HC1-3, staggered hippocampal wire electrodes

in wild-type mice and during wakefulness.<sup>9</sup> LEV reduced their density per sleep hour as well ( $P = .005$ ) (Figure 2B). Interestingly, LTG also decreased the number of cortical spikes per sleep hour ( $P < .05$ ). Whereas the LEV effect was dose-independent, the LTG effect was only seen with the larger dose (Figure 2E). In contrast, MEM increased the number of cortical spikes ( $P < .05$ ,  $n = 5$ ). In addition, the larger dose of MIN decreased cortical spikes ( $P < .05$ ,  $n = 5$ ). The absolute numbers of cortical spikes per total sleep time are shown in supplemental Figure S1.

SWDs in rodents are well characterized bilateral thalamo-cortical abnormal oscillations at 7 to 10 Hz that are present under light sleep.<sup>18</sup> Their prevalence in APP/PS1 mice is ~10 times higher than in wild-type littermates.<sup>19</sup> MEM drastically and dose-dependently increased the count of SWDs per sleep hour ( $P = .017$ ,  $n = 5$ ; Figure 2 C,F), while their number was decreased by LTG ( $P < .05$ ,  $n = 7$ ) and marginally by LEV ( $P = .07$ ,  $n = 6$ ). The other drugs did not significantly influence SWDs. The absolute numbers of spikes in SWDs per total sleep time are shown in supplemental Figure S1. In addition, examples of SWDs on vehicle versus MEM as given in Figure S3. MEM tended to increase both the number of spikes within an SWD and the amplitude of individual spikes.

### 3.3 | Sleep quality

AD is characterized by fragmented sleep, decreased slow-wave activity and substantial reduction in REM sleep duration.<sup>20</sup> Therefore, it is essential that drugs aimed to reduce sleep-related epileptiform activity do not further impair sleep quality. Of the effective drugs on epileptiform spiking, the smaller dose of LEV increased total sleep time ( $P = .003$ ,  $n = 7$ ), while IST ( $P < .05$ ,  $n = 7$ ) and MEM ( $P < .01$ ,  $n = 6$ ) both reduced total sleep time dose-dependently (Figure 4A). In con-

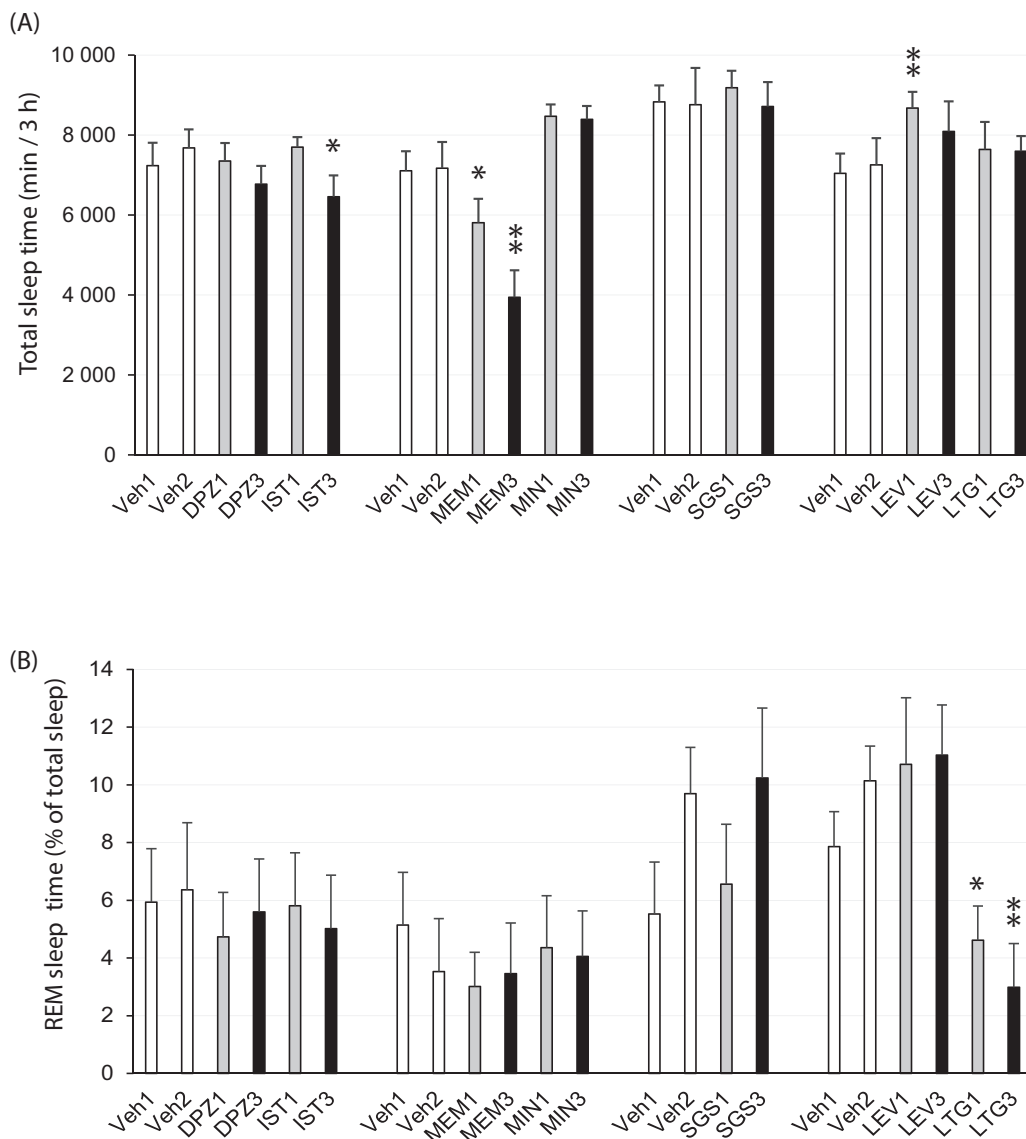
trast, LTG reduced the relative REM duration of total sleep time dose-dependently ( $P < .01$ ,  $n = 7$ ) without affecting the total sleep time. Other drugs had no significant effect on the sleep duration with the given doses. The drug effects on sleep are summarized in Figure 4.

## 4 | DISCUSSION

It is well established that epileptic activity is an essential feature of clinical AD, at least in the early disease stage.<sup>3</sup> Most of that activity appears to be subclinical, that is, it does not have motor manifestations and mainly happens during sleep, which makes its diagnosis particularly challenging. Recent overnight EEG studies concur that most epileptiform spiking localizes to the temporal lobe.<sup>4-6</sup> Despite the localization, the molecular basis of AD pathology is quite different from other known causes of temporal lobe epilepsy, which implies that the established drug treatment recommendations for temporal lobe epilepsy do not necessarily apply to AD-related epileptiform discharges. This is the first study to our knowledge that focuses on the therapeutic options on an AD-model specific for sleep-related epileptiform activity. The main finding of this study was that two anti-seizure drugs, LEV and LTG, which have been recommended for AD-related seizures based on their relatively good effect and tolerability,<sup>3</sup> differ dramatically in their effect on hippocampal giant spikes in the APP/PS1 AD mouse model.

LEV deviates from most anti-seizure drugs in that it does not block voltage-gated  $\text{Na}^+$ -channels but inhibits presynaptic  $\text{Ca}^{2+}$ -channels, thereby reducing glutamate release.<sup>21</sup> In vivo calcium imaging in APP/PS1 mice displays a zone of hyperactive neurons around amyloid plaques<sup>22</sup> and direct electrochemical recordings in vivo demonstrated increased glutamate release in the hippocampal CA1 region of the very same APPswe/PS1dE9 mice as in the present study around the time of first amyloid plaque formation.<sup>23</sup> We also found in our study mice that the majority of dystrophic neurites around amyloid plaques in the hippocampus near the verified electrode location (Figure 5A) stained strongly for the vesicular glutamate transporter, indicating that they concentrate glutamate in the neurotransmitter vesicles (Figure 5B). Therefore, it is conceivable that a treatment reducing presynaptic glutamate release would effectively counteract neuronal hyperactivity related to amyloid pathology. As MEM was without any protective effect against hippocampal giant spikes, one can conclude that the hyperactivation in hippocampal neurons due to increased glutamate release was mediated mainly via glutamate AMPA or kainate receptors.

On the other hand, the main mechanism of action of LTG is considered blockade of voltage-gated sodium channels as of many other anti-seizure drugs.<sup>24</sup> Interestingly, LTG was found to worsen seizures in the rare severe myoclonic epilepsy, aka Dravet syndrome,<sup>25</sup> which is caused by a mutation in the *SCN1A* gene coding for the  $\alpha$ -subunit of the Nav1.1 channel that is mainly responsible for action potential generation.<sup>26</sup> Nav1.1 closely relates to basic  $\text{A}\beta$  pathology in that its  $\beta$ -subunit is a substrate to BACE1.<sup>27</sup> BACE1 actions leads eventually to retention of Nav1.1 inside the cell.<sup>27</sup> Indeed, the levels of Nav1.1 protein were found to be reduced in APP transgenic mouse (hAPPJ20) and AD patient brains, especially in parvalbumin-positive

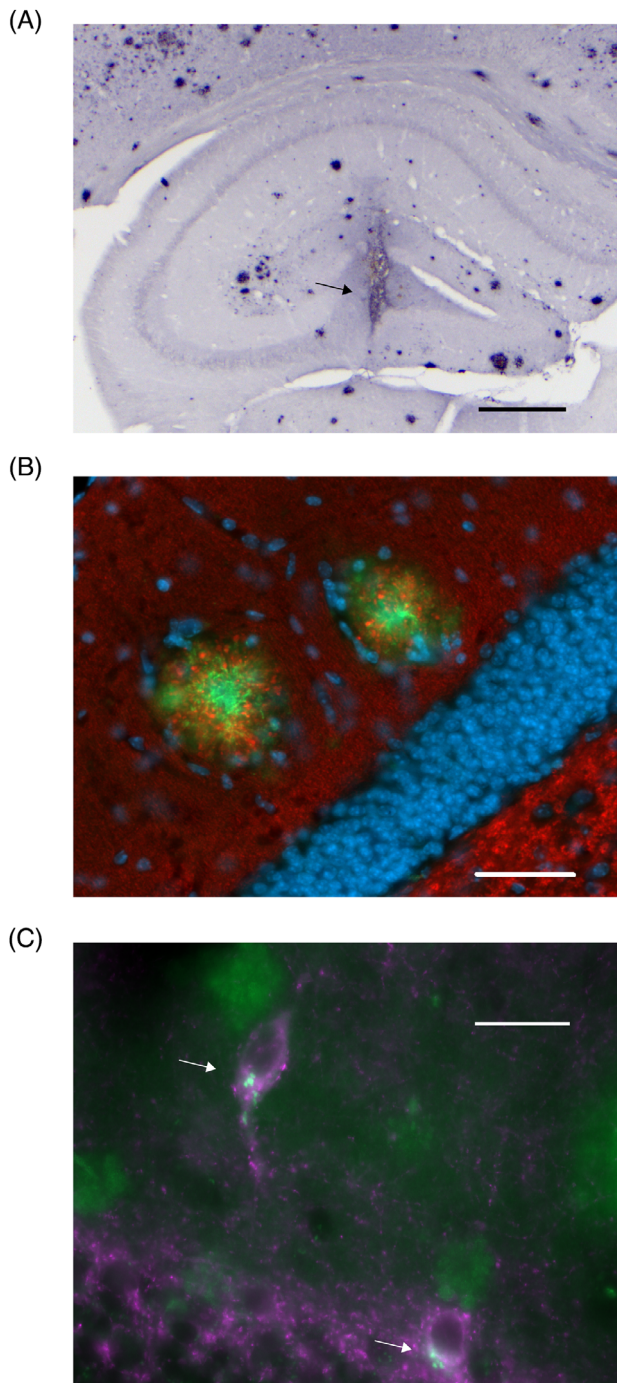


**FIGURE 4** Effect of the studied drugs on sleep quality. (A) Total sleep time per 3-h recording session under each drug treatment (gray columns low dose, black columns high dose) and corresponding vehicle injections (Veh1, Veh2, white columns). (B) Relative proportion of REM sleep time as % of total time in sleep. Asterisks indicate significant change from vehicle, \*  $P < 0.05$ , \*\*\*  $P < 0.001$ . Group means and SEMs are shown. Abbreviations: DPZ, donepezil; IST, istradefylline; LEV, levetiracetam; LTG, lamotrigine; MEM, memantine; MIN, minocycline; SGS, semagacestat; Veh, vehicle

interneurons.<sup>28</sup> These observations suggest that dystrophic neurites around amyloid plaques that are highly loaded with BACE1<sup>29</sup> may influence nearby interneurons and cause Nav1.1 loss on their surface. Furthermore, we found strong perinuclear BACE1 positivity in GAD67 positive interneurons in dentate gyrus of our mice (Figure 5C). If the Nav1.1 surface levels are already reduced, a small dose of Na<sup>+</sup>-channel blocker may disproportionately affect these interneurons and lead to altered excitation-inhibition balance. In support of this, the classic Na<sup>+</sup>-channel blocker phenytoin induced epileptiform activity in the very same hAPPJ20 mouse with reduced Nav1.1 levels in parvalbumin interneurons.<sup>28</sup> Most likely LTG acts through the same mechanism in our APP/PS1 mice. The data on wild-type mice also support a differential effect of LTG on GABAergic interneurons versus glutamatergic

principal neurons. A low LTG dose probably primarily blocked Na<sup>+</sup>-channels in interneurons resulting in disinhibition on principal neurons and giant spikes, whereas a 5-fold dose blocked these channels on both GABA and glutamate neurons, with no further spiking. Why then LTG induced epileptiform activity in the hippocampus but reduced that in the cortex remains to be studied, but may relate to different sensitivity of different neuronal subtypes to LTG. Since reduced Nav1.1 levels have been found also in AD brains, collectively these data call for caution in using LTG or other Na<sup>+</sup>-channel blockers for treating epileptiform discharges in AD patients.

Good news to all doctors treating AD patients with ACE inhibitors or MEM was that neither type of drug increased hippocampal giant spikes during sleep. Nevertheless, MEM marginally increased cortical



**FIGURE 5** (A) Example of distribution of amyloid plaques in the hippocampus at the level of the recording electrode (arrow). Human specific N-terminal amyloid staining (W02). Scale bar 0.5 mm. (B) Two neuritic plaques in the hippocampus (dentate gyrus, molecular layer) of an APP/PS1 mouse where the amyloid plaques (MoAb2 antibody, green) are surrounded by numerous swollen axon terminals that stain positive for vGlut1 antibody (red). Neural nuclei are stained with DAPI (blue). Scale bar 50  $\mu$ m. (C) Two GABAergic interneurons in the dentate gyrus of an APP/PS1 mouse stained with GAD67 antibody (purple) with the meshwork of their axon terminal amidst the dentate granule cells. Both neurons show strong perinuclear granular staining for BACE1 (light green, arrows). BACE1 positive dystrophic neurites around amyloid plaques are seen in the background (green). Scale bar 25  $\mu$ m

spiking and drastically increased the occurrence of SWDs. SWDs in certain inbred rat lines have been considered a model for childhood absence epilepsy.<sup>18</sup> However, electrographically human absence epilepsy with 3 Hz spike-waves complexes is distinct from the 7 to 10 Hz spike-waves in rodents. On the other hand, very similar abnormally slow mu rhythm as the rodent SWD has been documented in patients with Unverricht-Lundborg type progressive myoclonus epilepsy<sup>30</sup> but not in Alzheimer patients. Thus, the significance of this finding is unclear but the observation calls for further studies.

#### 4.1 | Conclusions

The purpose of this translational study was not to give treatment guidelines but rather give directions to future clinical trials on sleep-related epileptiform activity in early-stage AD patients. Of the seven different mechanisms of action represented by the chosen drug candidates, reduction of presynaptic glutamate release by LEV proved the most promising option. Not only was LEV the only drug that significantly reduced hippocampal giant spikes, but it also reduced cortical spikes. It increased the total sleep time without affecting the percentage of REM, which is a suitable profile for overnight use. On the other hand, it is also known to cause severe undesired effects such as psychiatric symptoms.<sup>3</sup> While preparing this manuscript, the results of a Phase 2 clinical trial on cognitive effects of a low-dose LEV (125 mg/kg twice daily) on AD patients were published.<sup>31</sup> Interestingly, cognitive improvement was reported in a subgroup with epileptiform activity but not among those without such activity. The active group did not report significantly more side effects than the placebo group. Although the study did not directly address the effect of LEV on epileptiform activity, the result is in line with these preclinical findings.

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#### CONFLICT OF INTEREST

The authors report no conflicts of interest related to this study.

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