Neurosteroidal modulation of in vitro epileptiform activity is enhanced in pilocarpine-treated epileptic rats

Zahra Shiri, Rochelle Herrington, Maxime Lévesque, and Massimo Avoli*

Montreal Neurological Institute and Department of Neurology & Neurosurgery, McGill University, 3801 University Street, Montréal, QC H3A 2B4, Canada

Abstract

We employed field potential recordings in brain slices obtained from pilocarpine-treated epileptic (4–5 weeks following a pilocarpine-induced status epilepticus) and age-matched, non-epileptic control (NEC) rats to establish the effects of the neurosteroid allotetrahydrodeoxycorticosterone (THDOC) on the epileptiform activity – including high frequency oscillations (HFOs; ripples: 80–200 Hz, fast ripples: 250–500 Hz) – induced by 4-aminopyridine (4AP) in piriform (PC) and entorhinal (EC) cortices. Both structures are highly susceptible to generate seizures and may also be involved in epileptogenesis. We found that THDOC application to pilocarpine-treated slices: (i) decreased interictal discharge frequency in PC while increasing it in EC; (ii) abolished ictal discharges in both areas in approx. one third of the experiments and reduced them in frequency and duration in the remaining experiments; and (iii) increased the occurrence of ripples and fast ripples associated to interictal events, and modified their pattern of occurrence during ictal discharges in both PC and EC. These effects were either weaker or absent in NEC tissue. Our results demonstrate that THDOC plays a structure-dependent modulatory role in epileptiform synchronization in the pilocarpine-treated epileptic rat brain where its actions are more pronounced than in NEC tissue. This evidence supports the application of neurosteroids as potential antiepileptic tools.

Keywords

Allotetrahydrodeoxycorticosterone; 4-Aminopyridine; Epileptogenesis; High frequency oscillations; Pilocarpine

Introduction

Mesial temporal lobe epilepsy (MTLE) is the most common form of partial epilepsy involving the hippocampus and parahippocampal structures such as the amygdala and the rhinal cortices (Gloor, 1997). Most of our current knowledge on the pathophysiology of this disease is based on animal models that reproduce a sequence of events similar to what is seen in MTLE patients. These events include an initial *status epilepticus* (SE) followed by a

*Corresponding author: massimo.avoli@mcgill.ca (M. Avoli).

Conflicts of interest

The authors declare that there is no conflict of interest.

seizure-free latent period of variable duration before the onset of spontaneously recurring seizures which identifies the start of the chronic period (Curia et al., 2008; Engel, 2001; Gloor, 1990; Lévesque and Avoli, 2013). In addition, MTLE patients and animals that underwent SE present with synaptic reorganization and neuronal cell loss (Bernard et al., 2000; Curia et al., 2008; Gorter et al., 2001; Shibley and Smith, 2002) in certain parahippocampal structures such as the piriform and entorhinal cortices (Cavalheiro, 1995; Covolan and Mello, 2000; Leite et al., 1990; Turski et al., 1983).

The piriform cortex (PC) is a highly excitable three-layered structure in the olfactory system involved in the generation and propagation of seizures (Haberly, 2001; Hoffman and Haberly, 1991). The PC projects to several limbic structures such as amygdala and the lateral entorhinal cortex (EC) (Haberly, 2001; Luskin and Price, 1983). EC neurons in turn project to the hippocampus, dentate gyrus, and subiculum (Witter et al., 1989). As already mentioned, the pilocarpine-induced SE causes a consistent pattern of neuronal damage in the PC and EC of the rodent brain (Scholl et al., 2013). These structures are often involved in limbic seizures in TLE patients (Gloor, 1997; Hoffman and Haberly, 1991; Roch et al., 2002).

Neurosteroids are a family of compounds synthesized from cholesterol in the brain (Baulieu, 1997) that control neuronal excitability by interacting with ion channels and receptors (Akk et al., 2009; Mellon and Griffin, 2002). One such molecule, allotetrahydrodeoxycorticosterone (THDOC), specifically modulates GABA_A receptor-mediated neurotransmission in a concentration-dependent manner; at lower concentrations (approx. 10 nM), THDOC interacts with GABA_A receptors while at higher concentrations (approx. 100 nM or more), it directly opens GABA_A channels (Belelli and Lambert, 2005). Following pilocarpine treatment, a transient upregulation of enzymes involved in THDOC synthesis in the hippocampus has been reported (Biagini et al., 2006; Pisu et al., 2008).

High frequency oscillations (HFOs) are thought to reflect the activity of dysfunctional neural networks and to represent better markers than interictal spikes to identify seizure onset zones (Jacobs et al., 2008, 2012; Jefferys et al., 2012a,b; Jiruska et al., 2010a,b; Urrestarazu et al., 2007; Zijlmans et al., 2009). Our group has previously characterized 4AP-induced epileptiform discharges in PC and EC (Hamidi et al., 2014) and has reported the effects of neurosteroids on such epileptiform activity and HFOs in the PC of healthy rats (Herrington et al., 2013). It remains however unknown whether these effects are maintained in the epileptic brain. Therefore, we examined the effects of THDOC on the interictal- and ictal-like (hereafter referred to as 'interictal' and 'ictal') discharges as well as on the associated HFOs generated during 4-aminopyridine (4AP) application by PC and EC neuronal networks in horizontal brain slices obtained from pilocarpine-treated epileptic and age matched, non-epileptic control (NEC) rats. By establishing the ability of neurosteroids to modify epileptiform synchronization in the epileptic tissue, we aim to support their potential use as antiepileptic compounds.

Materials and methods

All experimental procedures were carried out in compliance with guidelines from the Canadian Council on Animal Care and the McGill Animal Care Committee. All efforts were made to minimize the number of animals used and their suffering.

Preparation of pilocarpine-treated rats

SE was induced in 11 adult male Sprague Dawley rats (160–220 g) by a single intraperitoneal (i.p.) injection of the cholinergic agonist pilocarpine hydrochloride (380 mg/kg) (Bortel et al., 2010; Panuccio et al., 2010). A second half-dose of pilocarpine was given if SE did not occur within the first hour. To minimize discomfort caused by peripheral muscarinic receptor stimulation, rats were pre-treated with scopolamine methylnitrate (1 mg/kg, i.p.) 30 min prior to pilocarpine administration. Animal behavior was monitored and scored based on Racine's classification (Racine, 1972). SE (considered as a continuous stage 5 seizure) was terminated after 1 h with diazepam (5 mg/kg, s.c.) and ketamine (50 mg/kg, s.c.). NEC rats (n = 11) were injected with saline instead of pilocarpine. All rats were video monitored for 10 consecutive days starting 2 weeks following injection. Video recordings were examined off-line to verify that each pilocarpine-treated rat used in subsequent electrophysiological recordings experienced at least one spontaneous seizure indicating that they had entered the chronic phase. Electrophysiological experiments were carried out 4–5 weeks following pilocarpine or saline injection.

Preparation of brain slices for electrophysiological recordings

NEC and epileptic animals were decapitated under deep isoflurane anesthesia. Their brains were then quickly removed and chilled for 3 min in ice-cold (~4 °C) oxygenated (95% O₂, 5% CO₂) artificial cerebro-spinal fluid (ACSF) with the following composition (mM): 124 NaCl, 2 KCl, 2 CaCl₂, 2 MgSO₄, 1.25 KH₂PO₄, 26 NaHCO₃, 10 D-glucose (pH ~ 7.4). Horizontal brain slices (450 µm thick) were obtained with the use of a vibratome (VT1000s; Leica, Concord, Ontario, Canada) and quickly transferred to an interface chamber where they lay between warm (31–33 °C) ACSF (pH ~ 7.4, ~305 mOsm/kg) and humidified gas (95% O₂, 5% CO₂). Slices were allowed to recover for 90 min before the K⁺ channel blocker, 4AP (50 µM), was bath-applied at a rate of ~2 ml/min to induce epileptiform activity. Previous studies have shown that lower concentrations of THDOC failed to modulate 4AP-induced epileptiform activity; Therefore, we applied THDOC (5 µM) at a rate of ~2 ml/min for ~30 min (Herrington et al., 2013, 2014; Salazar et al., 2003). All pharmaceutical compounds were obtained from Sigma-Aldrich Canada, Ltd. (Oakville, Ontario, Canada).

Electrophysiological recordings

Field potential recordings were made with ACSF-filled glass pipettes (1B150F-4; World Precision Instruments, Sarasota, Florida, USA; tip diameter < 10 µm, resistance 5–10 MΩ) that were pulled with a Sutter P-97 puller (Sutter, Novato, CA, USA). Electrodes were placed in the posterior PC and in the lateral EC 60 min after 4AP application. Signals were fed to high-impedance amplifiers (Molecular Devices, Silicon Valley CA, USA), digitized (Digidata 1322A; Molecular Devices), acquired and stored using the pClamp software (8.0

and 9.0; Molecular Devices). Traces were sampled at 5 kHz and cut-off at 1 kHz. The frequency and duration of ictal and interictal discharges were analyzed offline using the CLAMPFIT 10.2 (Molecular Devices) software. In this analysis, events that lasted 4 s or longer were considered to be ictal events while events shorter than 4 s were deemed interictal events (Traub et al., 1996). Frequency was determined based on the number of events detected over the length of the recording. Event duration was measured as the length of time from the first deflection from baseline to its return to baseline. Amplitudes of interictal events were measured from peak to trough.

Detection and analysis of high-frequency oscillations

HFOs underlying interictal and ictal events were detected and analyzed using a multiparametric algorithm in MATLAB (The Mathworks, Natick, MA, USA). Recordings were downsampled to 2000 Hz, bandpass filtered in the 80–200 and 250–500 Hz frequency ranges using a finite impulse response filter; zero-phase digital filtering was used to avoid phase distortion. Filtered recordings were then normalized using the average RMS value of a 10-s artifact-free period, ranging from 50 to 60 s before the onset of the ictal discharge. To be considered as an HFO candidate, oscillatory events in each frequency band had to show at least four consecutive cycles having amplitude of 3 standard deviations above the mean. The time lag between two consecutive cycles had to be between 5 and 12.5 ms for ripples and between 2 and 4 ms for fast ripples (Lévesque et al., 2011, 2012; Salami et al., 2012). Overlapping events, which could be caused by filtering spikes (Bénar et al., 2010) were thus excluded from the analysis. In order to account for differences in duration, ictal events were analyzed in a time scale from 1 to 100. This period was then divided in three equal parts for statistical analysis. The rates of HFO occurrence were thus obtained for each interictal and ictal discharge in the EC and PC.

Statistical analysis

Measurements throughout the text are expressed as mean $\pm$ SEM. Data were compared using the Student's *t*-test. Ratios were tested for significance using Fisher's exact test. Results were considered significant if the *p*-value was less than 0.05. From here on, *n* refers to the number of slices studied.

Results

4AP-induced epileptiform activity in NEC and epileptic tissue

Pilocarpine was administered to 11 rats; a second half-dose was injected in 8 rats that did not experience SE within the hour. Data from animals that received a second half-dose of pilocarpine were statistically similar to animals that received a single dose and were thus pooled together for analysis. First, we set out to compare 4AP-induced epileptiform discharges in NEC and pilocarpine-treated epileptic brain slices. Typical interictal (asterisks) and ictal discharges recorded from PC and EC in these two types of brain tissue are shown in Figs. 1A and B. In PC of epileptic slices (*n* = 17 slices obtained from 11 rats), interictal events were less frequent but longer in duration in comparison to NEC slices (*n* = 15 slices obtained from 11 rats; *p* < 0.01). The frequency of ictal discharges was comparable between the two types of tissue; however, only 13 out of 17 epileptic slices (72%) generated ictal

activity and these events were shorter in duration ($p < 0.05$). The reduced ictogenic potential of 4AP in models of chronic epilepsy has been previously reported for the medial EC (Zahn et al., 2008, 2012). Here, we identified a similar effect in the PC since we recorded fewer interictal discharges as well as shorter ictal discharges (Fig. 1C).

In the EC of epileptic slices ($n = 14$ slices obtained from 7 rats), interictal events were less frequent and shorter in duration in comparison to NEC slices ($n = 10$ slices obtained from 6 rats; $p < 0.05$). The frequency of ictal discharges was comparable between the two types of tissue, although the duration was shorter in epileptic slices ($p < 0.01$). Overall, in the entorhinal cortex of epileptic slices, we recorded fewer interictal and ictal discharges with shorter durations in comparison to control slices (Fig. 1C). We previously reported that epileptiform discharges were less frequent and more robust in pilocarpine-treated EC compared to NEC tissue when recorded later in the chronic period (Panuccio et al., 2010).

HFOs associated with epileptiform activity in NEC and epileptic tissue

We also examined the occurrence of ripples and fast ripples during interictal events induced by 4AP in PC (Fig. 2A) and EC (Fig. 2B) of NEC and pilocarpine-treated epileptic tissue. In both structures, ripple and fast ripple rates were higher in epileptic tissue than in NEC slices ($p < 0.01$). In addition, fast ripple rates were higher than ripple rates in both tissue types ($p < 0.01$) (Figs. 2Ac, Bc).

We also analyzed HFO occurrence during normalized 4AP-induced ictal discharges in PC (Fig. 3Aa) and EC (Fig. 3Ba). We found that fast ripple rates were higher in the PC and EC of brain slices obtained from pilocarpine-treated epileptic rats than in those from NECs ($p < 0.05$; Figs. 3Ac, Bc). There was no difference in ripple rates in PC between control and epileptic tissue (Fig. 3Ab). In EC, ripples rates were higher at the onset of ictal discharges in epileptic slices compared to control ($p < 0.01$; Fig. 3Bb). In addition, there was a pronounced increase in ripple rates at the onset of ictal discharges in epileptic EC that declined throughout the event. On the contrary, we found that ripple rates increased throughout the discharge and occurred at higher rates towards the end of the event in the EC of NEC slices (Fig. 3Bb).

Effects of THDOC on 4AP-induced epileptiform activity in PC

Next, we analyzed the ability of THDOC to influence the 4AP-induced epileptiform discharges in PC of NEC and epileptic brain slices (Figs. 4A, B). THDOC did not affect the rate of occurrence of interictal discharges in NEC slices while decreasing it in epileptic slices ($p < 0.01$). Application of this neurosteroid also increased the duration of interictal events recorded from the PC in both control and epileptic slices ($p < 0.01$; Fig. 4C).

THDOC application completely abolished ictal events in 20% (3/15 slices) of the NEC slices and in 38.5% (5/13 slices) of epileptic slices. In those experiments in which ictal events continued to occur during THDOC treatment, we found that this compound did not change their frequency in NEC tissue ($n = 12$ slices from 9 rats) while decreasing it in epileptic tissue ($n = 8$ slices from 6 rats; $p < 0.05$). In addition, there was a decrease in ictal duration in NEC slices ($p < 0.05$) but no significant change in epileptic slices (Fig. 4C).

These results suggest that THDOC's ability to modulate epileptiform activity may be enhanced in epileptic tissue.

Effects of THDOC on 4AP-induced epileptiform activity in EC

The ability of THDOC to influence 4AP-induced epileptiform discharges in the EC of NEC and epileptic brain slices was then analyzed (Figs. 5A, B). THDOC had no effect on the interval of occurrence of interictal discharges in NEC slices while it increased their frequency in epileptic slices ($p < 0.01$). Adding THDOC to the bath did not affect the duration of interictal discharges in control tissue but increased this parameter in epileptic slices ($p < 0.01$; Fig. 5C).

THDOC application completely abolished ictal events in 10.0% (1/10 slices) of NEC and in 28.6% (4/14 slices) of epileptic slices. In those experiments in which ictal events continued to be recorded, THDOC did not change the frequency of ictal discharges in control tissue ($n = 9$ slices from 5 rats) while decreasing their occurrence in the epileptic tissue ($n = 10$ slices from 7 rats; $p < 0.01$). In addition, there was no change in ictal duration in NEC slices while a decrease was seen in epileptic slices ($p < 0.05$). Overall, THDOC application affected neither the frequency nor the duration of epileptiform discharges in NEC slices; while more frequent and longer interictal discharges as well as fewer and shorter ictal discharges were recorded in epileptic slices (Fig. 5C).

Effect of THDOC on HFOs underlying interictal discharges

We also analyzed the rate of occurrence of HFOs during interictal discharges before and after the addition of THDOC (Fig. 6). In the PC of NEC slices, THDOC did not affect the occurrence of ripples while decreasing the occurrence of fast ripples ($p < 0.01$) associated to interictal discharges. In the PC of pilocarpine-treated epileptic slices, THDOC increased both ripple ($p < 0.01$) and fast ripple ($p < 0.05$) occurrence during interictal events (Fig. 6A).

In the EC of NEC slices, THDOC affected neither ripple nor fast ripple occurrence during interictal discharges while in pilocarpine-treated epileptic slices it increased ripple occurrence ($p < 0.01$) without influencing fast ripple rates (Fig. 6B). Therefore, in both PC and EC of epileptic tissue, adding THDOC to the perfusion solution greatly increased the occurrence of ripples during interictal discharges.

Effect of THDOC on HFOs underlying ictal discharges

We also quantified the incidence of ripples and fast ripples during 4AP-induced ictal discharges in NEC and pilocarpine-treated epileptic tissue before and during THDOC application. In the PC of NEC slices, THDOC induced more ripples during the pre-ictal and post-ictal periods, but no significant difference was observed during the ictal period; in addition, fast ripple rates were significantly higher during the pre-ictal period in these experiments during THDOC (Fig. 7Aa). In the PC of epileptic animals, ripple and fast ripple occurrence peaked at the onset of the discharge under 4AP conditions; addition of THDOC induced in these experiments higher rates of ripples and fast ripples during the terminal phase of the ictal period (Fig. 7Ab). THDOC did not change the number of ripples but decreased the number of fast ripples associated with ictal discharges in control tissue. This

neurosteroid did not change the number of HFO events during ictal discharges in epileptic tissue (Table 2).

In the EC of NEC slices, THDOC addition reduced ripple rates while increasing the occurrence of fast ripples during the ictal period (Fig. 7Ba). In the EC of epileptic tissue, THDOC induced higher rates of ripples and fast ripples during the late phase of the ictal discharges (Fig. 7Bb). Therefore in both PC and EC of pilocarpine-treated epileptic slices, THDOC changed the timing of HFO occurrence during ictal discharges thus suggesting that THDOC modulation of HFOs associated to ictal activity is altered in the epileptic brain. THDOC did not change the number of ripples or fast ripples associated with ictal discharges in control tissue. In epileptic tissue, it decreased the number of ripple and fast ripples during ictal discharges (Table 2).

Discussion

In this study, we used the pilocarpine model of TLE along with field potential recordings from PC and EC of horizontal rat brain slices maintained in vitro to investigate the effect of THDOC on the epileptiform discharges generated by control and epileptic tissues. Pilocarpine-induced SE increases the plasticity of GABA_A receptors presumably leading to a reduction in GABA_A receptor-mediated inhibition (Joshi and Kapur, 2012). Here, we have reported that THDOC application to pilocarpine-treated slices: (i) decreased interictal discharge frequency in the PC while increasing it in EC; (ii) abolished ictal discharges in both areas in approximately one third of the experiments and reduced them in frequency and duration in the remaining experiments; and (iii) increased the occurrence of ripples and fast ripples associated to interictal events, and modified their pattern of occurrence during ictal discharges in both PC and EC. In contrast, as summarized in Tables 1 and 2, these effects were often weaker or even absent in NEC tissue.

4AP-induced epileptiform discharges in control and epileptic tissue

The K⁺ channel blocker 4AP, is a common convulsant used in vivo (Fragoso-Veloz et al., 1990; Lévesque et al., 2013; Mihály et al., 1990) and in vitro (Hamidi et al., 2014; Herrington et al., 2013; Panuccio et al., 2012) to induce epileptiform synchronization by enhancing both GABAergic and glutamatergic neurotransmission (Perreault and Avoli, 1991; Rutecki et al., 1987). It has been reported that brain slices obtained from epileptic rats are characterized by a reduced ability to generate ictal discharges in vitro during 4AP treatment when recordings are performed in the medial EC (Zahn et al., 2012). They showed that 50 μM 4AP could induce seizure-like events in the medial EC in only 20% of pilocarpine-treated slices. In contrast, we have previously reported that epileptic slices generate robust ictal-like activity in response to 4AP treatment (Pannuccio et al., 2010). In our present experiments as well, we observed seizure-like events in all EC and in 72% of PC recordings from epileptic slices using the same concentration of 4AP. The absence of ictal activity in 28% of PC recordings may mirror the SE-induced changes that, as suggested by Zahn et al. (2012), should alter the expression of 4AP-sensitive potassium channels. Therefore, the only difference is that we recorded fewer but longer interictal discharges and

shorter ictal events in PC as well as fewer interictal and ictal discharges with shorter durations in the EC in comparison to control slices.

Altered HFO pattern in epileptic tissue

HFOs are one form of neuronal synchronization seen in TLE as well as in other epileptic disorders (Perucca et al., 2014). Pathological HFOs arise from abnormal neural networks and fall into two categories of ripples or fast ripples. Ripples represent population inhibitory post-synaptic potentials generated by principal cells that are entrained by synchronously active interneuronal networks (Buzsáki et al., 1992; Ylinen et al., 1995). Fast ripples reflect synchronous firing of abnormally active principal cells (Bragin et al., 2011; Dzhala and Staley, 2003; Engel et al., 2009). It has been shown that SE induces tissue damage leading to synaptic reorganization (Gorter et al., 2001; Sutula et al., 1989), loss of specific interneuron subtypes (Bernard et al., 2000; Maglóczy and Freund, 2005), and alteration in GABAergic inhibition (Brooks-Kayal et al., 1998; Joshi and Kapur, 2012; Sloviter, 1987). Because of these cellular changes and the resulting abnormal neural activity, we expected to detect more HFOs in post-SE slices.

Indeed, in both PC and EC, ripple and fast ripple rates associated with interictal discharges were higher in epileptic tissue. In addition, fast ripples occurred at higher rates during ictal discharges in both structures. This increase in HFO occurrence may be due to a deficit in GABAergic inhibition (Bernard et al., 2000) due to loss of specific inter-neuron subtypes which leads to loss of inhibitory synapses onto principal cells. We also found a significant increase in ripple rates at the onset of ictal discharges in epileptic EC that slowly declines throughout the discharge; in contrast, we found in EC of NEC tissue that ripple rates increase throughout the discharge and occur at higher rates towards the end of the event. These differences may shed some light on the different mechanisms of ictogenesis in NEC and epileptic tissue. Once more, synaptic reorganization and alterations in GABAergic inhibition following SE may be responsible for this abnormal interneuronal activity at the onset of ictal discharges.

THDOC modulation of 4AP-induced epileptiform discharges

GABA_A receptors vary in terms of subunit composition. The δ -subunit containing receptors are mainly extrasynaptic and are the primary mediators of tonic inhibition (Belelli and Lambert, 2005; Glykys et al., 2008). In addition, δ -subunit containing GABA_A receptors are the main site of neurosteroid action (Wohlfarth et al., 2002). However, it has been suggested that this particular subtype is downregulated in TLE, compromising neurosteroidal enhancement of tonic inhibition (Walker and Kullmann, 2012). In fact, one study reported that physiological concentrations of THDOC (10 nM) were less effective in modulating excitability in epileptic animals (Peng et al., 2004).

Neurosteroids can exert effects even in the nanomolar concentration range; however, these studies are usually conducted in the absence of convulsive drugs such as 4AP. For THDOC to directly activate GABA_A receptor currents, concentrations greater than 1 μ M have been reported (Reddy and Rogawski, 2002). In a study conducted by Salazar et al. (2003) it was demonstrated that neurosteroid concentrations of 90 μ M were required to attenuate

epileptiform activity induced by 4AP. Recently, Herrington et al. (2014) found that lower concentrations of THDOC (5 μ M) could abolish ictal events while interictal events persisted. Therefore, we decided that 5 μ M would be an appropriate concentration to use for our study. Potentiating GABAergic conductances plays an important role in controlling the onset of epileptiform activity but higher “activating” concentrations may be required in vivo to abolish seizures in epileptic networks.

Here we have shown that in the presence of 5 μ M THDOC, PC ictal discharges were completely abolished in more than one third of the epileptic slices and in the remaining slices, the frequency of these events was less than one half. In EC of epileptic slices, THDOC administration resulted in more frequent and longer interictal discharges while reducing both the frequency and duration of ictal discharges. Interestingly, THDOC affected neither the frequency nor the duration of epileptiform discharges in EC of NEC slices. Therefore these results suggest that at high, presumably pharmacologically relevant, concentrations the ability of THDOC to modulate epileptiform activity is enhanced in both the piriform and entorhinal cortices of epileptic tissue.

Although PC and EC are anatomically connected, there is much evidence suggesting that they are functionally segregated (Uva et al., 2013). These cortical structures differ in terms of cell composition and connectivity and thus differ in the way interneurons innervate various pyramidal cell compartments and consequently the way they participate in network oscillations (Gavrilovici et al., 2012; Middleton et al., 2008). In addition, pilocarpine treatment induces interneuronal cell loss and reorganization differently in each cortical structure, which may result in various degrees of reduced inhibitory synaptic inputs. For instance, in the EC, pilocarpine-induced SE results in nearly 33% loss of GABAergic interneurons while a more robust damage has been reported in PC (Kumar and Buckmaster, 2006; Turski et al., 1984). These differences could account for the differential modulation of epileptiform discharges by THDOC reported here.

THDOC modulation of HFOs in epileptic tissue

As previously shown by our group, THDOC administration reduced HFO occurrence in PC of control tissue (Herrington et al., 2013). We have found that in both PC and EC of epileptic tissue, adding THDOC to the bathing solution greatly increased the occurrence of ripples during interictal discharges. It is interesting to note that in both structures, when analyzed in the pilocarpine-treated epileptic tissue, we detected approximately twice as many fast ripples than ripples. This finding is in line with what was reported by Staba et al. (2007) who showed a correlation between higher ratios of fast ripple to ripple rates and reduced hippocampal volumes as occurs post-SE. As already mentioned, it has been shown that SE induces cell loss and synaptic reorganization promoting fast ripple generation.

In the piriform and entorhinal cortices of epileptic tissue, bath application of THDOC changed the timing of HFO occurrence from the onset of the ictal discharge to the offset of the latter. In fact, there seemed to be a progressive increase in HFO occurrence throughout the discharge. Thus these data suggest that THDOC modulation of epileptiform discharges is altered in post-SE slices, presumably due to the changes in GABA_A receptor composition as

well as the reduced inhibitory synaptic input (Joshi and Kapur, 2012; Kumar and Buckmaster, 2006; Walker and Kullmann, 2012).

Conclusions

We have found that neurosteroids at high concentrations modulate epileptiform synchronization in a structure-dependent manner in NEC and even more so in pilocarpine-treated epileptic rat brains. Our evidence supports, therefore, the use of neurosteroids as potential anti-epileptic tools (Reddy and Rogawski, 2012). However, one must keep in mind that although in vitro preparations provide a rapid, flexible, and accessible approach to studying neurosteroid modulation of epileptiform activity, such preparations are oversimplified in terms of connectivity (Heinemann and Staley, 2014). This reduced connectivity is often compensated for by application of acute convulsive agents in vitro. Slice preparations also lack a blood brain barrier, which should be considered when discussing novel pharmacological approaches. However, it is well known that steroids are highly lipophilic and can readily cross the blood brain barrier in the intact brain. Another limitation of in vitro experiments is that the long-term loss of efficacy and tolerance development cannot be evaluated. In vivo studies in mice however have shown that anticonvulsant tolerance is not obtained with neurosteroids (Kokate et al., 1999). Therefore, although reliable, the results presented here cannot be fully translated to the in vivo condition of epilepsy (see: Frye, 1995; Kokate et al., 1994).

Acknowledgments

This study was supported by the Canadian Institutes of Health Research (CIHR grants 8109 and 74609 to MA). The funding agency had no role in the study design, data collection, data analysis, decision to publish, and preparation of the manuscript.

Abbreviations

NEC	non-epileptic control
THDOC	allotetrahydrodeoxycorticosterone
4AP	4-aminopyridine
HFO	high frequency oscillations
PC	piriform cortex
EC	entorhinal cortex
MTLE	mesial temporal lobe epilepsy
SE	status epilepticus
GABA_A	gamma-aminobutyric acid receptor type A

References

- Akk G, Covey DF, Evers AS, Steinbach JH, Zorumski CF, Mennerick S. The influence of the membrane on neurosteroid actions at GABA(A) receptors. *Psychoneuroendocrinology*. 2009; 34(Suppl 1):S59–S66. [PubMed: 19541427]
- Baulieu EE. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. *Recent Prog Horm Res*. 1997; 52:1–32.
- Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABAA receptor. *Nat Rev Neurosci*. 2005; 6:565–575. [PubMed: 15959466]
- Bénar CG, Chauvière L, Bartolomei F, Wendling F. Pitfalls of high-pass filtering for detecting epileptic oscillations: a technical note on “false” ripples. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol*. 2010; 121:301–310.
- Bernard C, Cossart R, Hirsch JC, Esclapez M, Ben-Ari Y. What is GABAergic inhibition? How is it modified in epilepsy? *Epilepsia*. 2000; 41(Suppl 6):S90–S95. [PubMed: 1099527]
- Biagini G, Baldelli E, Longo D, Pradelli L, Zini I, Rogawski MA, Avoli M. Endogenous neurosteroids modulate epileptogenesis in a model of temporal lobe epilepsy. *Exp Neurol*. 2006; 201:519–524. [PubMed: 16780839]
- Bortel A, Lévesque M, Biagini G, Gotman J, Avoli M. Convulsive status epilepticus duration as determinant for epileptogenesis and interictal discharge generation in the rat limbic system. *Neurobiol Dis*. 2010; 40:478–489. [PubMed: 20682341]
- Bragin A, Benassi SK, Kheiri F, Engel J. Further evidence that pathologic high-frequency oscillations are bursts of population spikes derived from recordings of identified cells in dentate gyrus. *Epilepsia*. 2011; 52:45–52.
- Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat Med*. 1998; 4:1166–1172. [PubMed: 9771750]
- Buzsáki G, Horváth Z, Urioste R, Hetke J, Wise K. High-frequency network oscillation in the hippocampus. *Science*. 1992; 256:1025–1027. [PubMed: 1589772]
- Cavalheiro EA. The pilocarpine model of epilepsy. *Ital J Neurol Sci*. 1995; 16:33–37. [PubMed: 7642349]
- Covolan L, Mello LEAM. Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. *Epilepsy Res*. 2000; 39:133–152. [PubMed: 10759302]
- Curia G, Longo D, Biagini G, Jones RSG, Avoli M. The pilocarpine model of temporal lobe epilepsy. *J Neurosci Methods*. 2008; 172:143–157. [PubMed: 18550176]
- Dzhala VI, Staley KJ. Transition from interictal to ictal activity in limbic networks in vitro. *J Neurosci*. 2003; 23:7873–7880. [PubMed: 12944517]
- Engel J Jr. Mesial temporal lobe epilepsy: what have we learned? *Neurosci Rev J Bringing Neurobiol Neurol Psychiatry*. 2001; 7:340–352.
- Engel J Jr, Bragin A, Staba R, Mody I. High-frequency oscillations: what is normal and what is not? *Epilepsia*. 2009; 50:598–604. [PubMed: 19055491]
- Fragoso-Veloz J, Massieu L, Alvarado R, Tapia R. Seizures and wet-dog shakes induced by 4-aminopyridine, and their potentiation by nifedipine. *Eur J Pharmacol*. 1990; 178:275–284. [PubMed: 2340861]
- Frye CA. The neurosteroid 3 α ,5 α -THP has antiseizure and possible neuroprotective effects in an animal model of epilepsy. *Brain Res*. 1995; 696:113–120. [PubMed: 8574658]
- Gavrilovici C, Pollock E, Everest M, Poulter MO. The loss of interneuron functional diversity in the piriform cortex after induction of experimental epilepsy. *Neurobiol Dis*. 2012; 48:317–328. [PubMed: 22801084]
- Gloor P. Experiential phenomena of temporal lobe epilepsy facts and hypotheses. *Brain*. 1990; 113:1673–1694. [PubMed: 2276040]
- Gloor, P. *The Temporal Lobe and Limbic System*. Oxford University Press; New York: 1997.
- Glykys J, Mann EO, Mody I. Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? *J Neurosci Off J Soc Neurosci*. 2008; 28:1421–1426.

- Gorter JA, van Vliet EA, Aronica E, Lopes da Silva FH. Progression of spontaneous seizures after status epilepticus is associated with mossy fibre sprouting and extensive bilateral loss of hilar parvalbumin and somatostatin-immunoreactive neurons. *Eur J Neurosci.* 2001; 13:657–669. [PubMed: 11207801]
- Haberly LB. Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. *Chem Senses.* 2001; 26:551–576. [PubMed: 11418502]
- Hamidi S, Lévesque M, Avoli M. Epileptiform synchronization and high-frequency oscillations in brain slices comprising piriform and entorhinal cortices. *Neuroscience.* 2014
- Heinemann, U., Staley, KJ. What is the clinical relevance of in vitro epileptiform activity?. In: Scharfman, HE., Buckmaster, PS., editors. *Issues in Clinical Epileptology: A View from the Bench.* Springer; Netherlands: 2014. p. 25–41.
- Herrington R, Lévesque M, Avoli M. Neurosteroids modulate epileptiform activity and associated high-frequency oscillations in the piriform cortex. *Neuroscience.* 2013; 256:467–477. [PubMed: 24157930]
- Herrington R, Lévesque M, Avoli M. Neurosteroids differentially modulate fast and slow interictal discharges in the hippocampal CA3 area. *Eur J Neurosci.* 2014; 41(3):379–389. [PubMed: 25471484]
- Hoffman WH, Haberly LB. Bursting-induced epileptiform EPSPs in slices of piriform cortex are generated by deep cells. *J Neurosci Off J Soc Neurosci.* 1991; 11:2021–2031.
- Jacobs J, LeVan P, Chander R, Hall J, Dubeau F, Gotman J. Interictal high-frequency oscillations (80–500 Hz) are an indicator of seizure onset areas independent of spikes in the human epileptic brain. *Epilepsia.* 2008; 49:1893–1907. [PubMed: 18479382]
- Jacobs J, Staba R, Asano E, Otsubo H, Wu JY, Zijlmans M, Mohamed I, Kahane P, Dubeau F, Navarro V, et al. High-frequency oscillations (HFOs) in clinical epilepsy. *Prog Neurobiol.* 2012; 98:302–315. [PubMed: 22480752]
- Jefferys JGR, Menendez de la Prida L, Wendling F, Bragin A, Avoli M, Timofeev I, Lopes da Silva FH. Mechanisms of physiological and epileptic HFO generation. *Prog Neurobiol.* 2012a; 98:250–264. [PubMed: 22420980]
- Jefferys, JGR., Jiruska, P., de Curtis, M., Avoli, M. Limbic network synchronization and temporal lobe epilepsy. In: Noebels, JL., Avoli, M., Rogawski, MA., Olsen, RW., Delgado-Escueta, AV., editors. *Jasper's Basic Mechanisms of the Epilepsies.* National Center for Biotechnology Information (US); Bethesda (MD): 2012b.
- Jiruska P, Powell AD, Chang WC, Jefferys JGR. Electrographic high-frequency activity and epilepsy. *Epilepsy Res.* 2010a; 89:60–65. [PubMed: 20031373]
- Jiruska P, Csicsvari J, Powell AD, Fox JE, Chang WC, Vreugdenhil M, Li X, Palus M, Bujan AF, Dearden RW, et al. High-frequency network activity, global increase in neuronal activity, and synchrony expansion precede epileptic seizures in vitro. *J Neurosci Off J Soc Neurosci.* 2010b; 30:5690–5701.
- Joshi, S., Kapur, J. GABAA receptor plasticity during status epilepticus. In: Noebels, JL., Avoli, M., Rogawski, MA., Olsen, RW., Delgado-Escueta, AV., editors. *Jasper's Basic Mechanisms of the Epilepsies.* National Center for Biotechnology Information (US); Bethesda (MD): 2012.
- Kokate TG, Svensson BE, Rogawski MA. Anticonvulsant activity of neurosteroids: correlation with gamma-aminobutyric acid-evoked chloride current potentiation. *J Pharmacol Exp Ther.* 1994; 270:1223–1229. [PubMed: 7932175]
- Kokate TG, Juhng KN, Kirkby RD, Llamas J, Yamaguchi S, Rogawski MA. Convulsant actions of the neurosteroid pregnenolone sulfate in mice. *Brain Res.* 1999; 831:119–124. [PubMed: 10411990]
- Kumar SS, Buckmaster PS. Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. *J Neurosci Off J Soc Neurosci.* 2006; 26:4613–4623.
- Leite JP, Bortolotto ZA, Cavalheiro EA. Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy. *Neurosci Biobehav Rev.* 1990; 14:511–517. [PubMed: 2287490]
- Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. *Neurosci Biobehav Rev.* 2013; 37:2887–2899. [PubMed: 24184743]

- Lévesque M, Bortel A, Gotman J, Avoli M. High-frequency (80–500 Hz) oscillations and epileptogenesis in temporal lobe epilepsy. *Neurobiol Dis.* 2011; 42:231–241. [PubMed: 21238589]
- Lévesque M, Salami P, Gotman J, Avoli M. Two seizure-onset types reveal specific patterns of high-frequency oscillations in a model of temporal lobe epilepsy. *J Neurosci Off J Soc Neurosci.* 2012; 32:13264–13272.
- Lévesque M, Salami P, Behr C, Avoli M. Temporal lobe epileptiform activity following systemic administration of 4-aminopyridine in rats. *Epilepsia.* 2013; 54:596–604. [PubMed: 23521339]
- Luskin MB, Price JL. The topographic organization of associational fibers of the olfactory system in the rat, including centrifugal fibers to the olfactory bulb. *J Comp Neurol.* 1983; 216:264–291. [PubMed: 6306065]
- Maglóczy Z, Freund TF. Impaired and repaired inhibitory circuits in the epileptic human hippocampus. *Trends Neurosci.* 2005; 28:334–340. [PubMed: 15927690]
- Mellon SH, Griffin LD. Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab.* 2002; 13:35–43. [PubMed: 11750861]
- Middleton S, Jalics J, Kispersky T, Lebeau FEN, Roopun AK, Kopell NJ, Whittington MA, Cunningham MO. NMDA receptor-dependent switching between different gamma rhythm-generating microcircuits in entorhinal cortex. *Proc Natl Acad Sci U S A.* 2008; 105:18572–18577. [PubMed: 18997013]
- Mihály A, Bencsik K, Solymosi T. Naltrexone potentiates 4-aminopyridine seizures in the rat. *J Neural Transm Gen Sect.* 1990; 79:59–67. [PubMed: 2153388]
- Panuccio G, D'Antuono M, de Guzman P, De Lannoy L, Biagini G, Avoli M. In vitro ictogenesis and parahippocampal networks in a rodent model of temporal lobe epilepsy. *Neurobiol Dis.* 2010; 39:372–380. [PubMed: 20452424]
- Panuccio G, Sanchez G, Lévesque M, Salami P, de Curtis M, Avoli M. On the ictogenic properties of the piriform cortex in vitro. *Epilepsia.* 2012; 53:459–468. [PubMed: 22372627]
- Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the delta subunit of the GABAA receptor in a mouse model of temporal lobe epilepsy. *J Neurosci Off J Soc Neurosci.* 2004; 24:8629–8639.
- Perreault P, Avoli M. Physiology and pharmacology of epileptiform activity induced by 4-aminopyridine in rat hippocampal slices. *J Neurophysiol.* 1991; 65:771–785. [PubMed: 1675671]
- Perucca P, Dubeau F, Gotman J. Intracranial electroencephalographic seizure-onset patterns: effect of underlying pathology. *Brain.* 2014; 137:183–196. [PubMed: 24176980]
- Pisu MG, Mostallino MC, Dore R, Mura ML, Maciocco E, Russo E, De Sarro G, Serra M. Neuroactive steroids and GABAA receptor plasticity in the brain of the WAG/Rij rat, a model of absence epilepsy. *J Neurochem.* 2008; 106:2502–2514. [PubMed: 18624910]
- Racine RJ. Modification of seizure activity by electrical stimulation. II Motor seizure. *Electroencephalogr Clin Neurophysiol.* 1972; 32:281–294. [PubMed: 4110397]
- Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABAA receptor function and seizure susceptibility. *J Neurosci.* 2002; 22:3795–3805. [PubMed: 11978855]
- Reddy, DS., Rogawski, MA. Neurosteroids — endogenous regulators of seizure susceptibility and role in the treatment of epilepsy. In: Noebels, JL, Avoli, M, Rogawski, MA, Olsen, RW., Delgado-Escueta, AV., editors. *Jasper's Basic Mechanisms of the Epilepsies*. National Center for Biotechnology Information (US); Bethesda (MD): 2012.
- Roch C, Leroy C, Nehlig A, Namer IJ. Predictive value of cortical injury for the development of temporal lobe epilepsy in 21-day-old rats: an MRI approach using the lithium–pilocarpine model. *Epilepsia.* 2002; 43:1129–1136. [PubMed: 12366725]
- Rutecki PA, Lebeda FJ, Johnston D. 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. *J Neurophysiol.* 1987; 57:1911–1924. [PubMed: 3037040]
- Salami P, Lévesque M, Gotman J, Avoli M. A comparison between automated detection methods of high-frequency oscillations (80–500 Hz) during seizures. *J Neurosci Methods.* 2012; 211:265–271. [PubMed: 22983173]

- Salazar P, Tapia R, Rogawski MA. Effects of neurosteroids on epileptiform activity induced by picrotoxin and 4-aminopyridine in the rat hippocampal slice. *Epilepsy Res.* 2003; 55:71–82. [PubMed: 12948618]
- Scholl EA, Dudek FE, Ekstrand JJ. Neuronal degeneration is observed in multiple regions outside the hippocampus after lithium pilocarpine-induced status epilepticus in the immature rat. *Neuroscience.* 2013; 252:45–59. [PubMed: 23896573]
- Shibley H, Smith BN. Pilocarpine-induced status epilepticus results in mossy fiber sprouting and spontaneous seizures in C57BL/6 and CD-1 mice. *Epilepsy Res.* 2002; 49:109–120. [PubMed: 12049799]
- Sloviter RS. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science.* 1987; 235:73–76. [PubMed: 2879352]
- Staba RJ, Frighetto L, Behnke EJ, Mathern GW, Fields T, Bragin A, Ogren J, Fried I, Wilson CL, Engel J Jr. Increased fast ripple to ripple ratios correlate with reduced hippocampal volumes and neuron loss in temporal lobe epilepsy patients. *Epilepsia.* 2007
- Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol.* 1989; 26:321–330. [PubMed: 2508534]
- Traub RD, Borck C, Colling SB, Jefferys JG. On the structure of ictal events in vitro. *Epilepsia.* 1996; 37:879–891. [PubMed: 8814102]
- Turski WA, Czuczwar SJ, Kleinrok Z, Turski L. Cholinomimetics produce seizures and brain damage in rats. *Experientia.* 1983; 39:1408–1411. [PubMed: 6140182]
- Turski WA, Cavalheiro EA, Bortolotto ZA, Mello LM, Schwarz M, Turski L. Seizures produced by pilocarpine in mice: a behavioral, electroencephalographic and morphological analysis. *Brain Res.* 1984; 321:237–253. [PubMed: 6498517]
- Urrestarazu E, Chander R, Dubeau F, Gotman J. Interictal high-frequency oscillations (100–500 Hz) in the intracerebral EEG of epileptic patients. *Brain J Neurol.* 2007; 130:2354–2366.
- Uva L, Trombin F, Carriero G, Avoli M, de Curtis M. Seizure-like discharges induced by 4-aminopyridine in the olfactory system of the in vitro isolated guinea pig brain. *Epilepsia.* 2013; 54:605–615. [PubMed: 23505998]
- Walker, MC., Kullmann, DM. Tonic GABAA receptor-mediated signaling in epilepsy. In: Noebels, JL, Avoli, M, Rogawski, MA, Olsen, RW., Delgado-Escueta, AV., editors. *Jasper's Basic Mechanisms of the Epilepsies*. National Center for Biotechnology Information (US); Bethesda (MD): 2012.
- Witter MP, Groenewegen HJ, Lopes da Silva FH, Lohman AH. Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. *Prog Neurobiol.* 1989; 33:161–253. [PubMed: 2682783]
- Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. *J Neurosci Off J Soc Neurosci.* 2002; 22:1541–1549.
- Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A, Buzsaki G. Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci.* 1995; 15:30–46. [PubMed: 7823136]
- Zahn RK, Tolner EA, Derst C, Gruber C, Veh RW, Heinemann U. Reduced ictogenic potential of 4-aminopyridine in the perirhinal and entorhinal cortex of kainate-treated chronic epileptic rats. *Neurobiol Dis.* 2008; 29:186–200. [PubMed: 17942314]
- Zahn RK, Liotta A, Kim S, Sandow N, Heinemann U. Reduced ictogenic potential of 4-aminopyridine in the hippocampal region in the pilocarpine model of epilepsy. *Neurosci Lett.* 2012; 513:124–128. [PubMed: 22342924]
- Zijlmans M, Jacobs J, Zelman R, Dubeau F, Gotman J. High frequency oscillations and seizure frequency in patients with focal epilepsy. *Epilepsy Res.* 2009; 85:287–292. [PubMed: 19403269]

Fig. 1.

Characterization of 4AP-induced epileptiform activity in control and epileptic slices. (A) Interictal (asterisks) and ictal discharges recorded from PC of non-epileptic control (NEC) and epileptic (pilocarpine-treated) slices following the addition of 4AP. (B) Interictal (asterisks) and ictal discharges recorded from EC of non-epileptic control (NEC) and epileptic (pilocarpine-treated) slices following the addition of 4AP. (C) PC ($n = 15$ for NEC; $n = 17$ for pilocarpine-treated) and EC ($n = 9$ for NEC; $n = 14$ for pilocarpine-treated) interictal events quantified in terms of interval (top panel) and duration (second panel); PC ($n = 15$ for NEC; $n = 13$ for pilocarpine-treated) and EC ($n = 10$ for NEC; $n = 14$ for pilocarpine-treated) ictal-like events quantified in terms of interval (third panel) and duration (bottom panel) (* $p < 0.05$; ** $p < 0.01$).

Fig. 2. HFOs underlying 4AP-induced interictal events. (A) Field recordings showing a representative interictal event in PC of epileptic slices (a) co-occurring with a ripple; and (b) another event co-occurring with a fast ripple; (c) bar graphs quantifying the number of interictal events associated with either ripples or fast ripples in PC of control (white) and epileptic (black) slices (NEC: $n = 15$, events = 3812; pilocarpine-treated: $n = 17$, events = 921). (B) LFP showing a representative interictal event in EC of epileptic slices (a) co-occurring with a ripple; and (b) another event co-occurring with a fast ripple; (c) bar graphs quantifying the number of interictal events associated with either ripples or fast ripples in EC of control (white) and epileptic (black) slices (NEC: $n = 9$, events = 487; pilocarpine-treated: $n = 14$, events = 864; ** $p < 0.01$).

Fig. 3. HFOs underlying 4AP-induced ictal discharges. (A) LFP showing (a) an ictal event in PC of epileptic slices with filtered traces showing the associated ripples and fast ripples; rates of (b) ripple and (c) fast ripple occurrence in the pre-ictal, ictal, and post-ictal phases (NEC: $n = 15$, events = 114; pilocarpine-treated: $n = 13$, events = 74). (B) LFP showing (a) an ictal event in EC of epileptic slices with filtered traces showing the associated ripples and fast ripples; rates of (b) ripple and (c) fast ripple occurrence in the pre-ictal, ictal, and post-ictal phases (NEC: $n = 10$, events = 37; pilocarpine-treated: $n = 14$, events = 62; * indicates $p < 0.05$; ** $p < 0.01$).

Fig. 4.

Effect of THDOC on 4AP-induced epileptiform activity in PC. (A) Interictal (asterisks) and ictal discharges recorded from PC of non-epileptic control (NEC) slices in 4AP and following the addition of THDOC. (B) Interictal (asterisks) and ictal discharges recorded from PC of epileptic (pilocarpine-treated) slices in 4AP and following bath application of THDOC. (C) PC interictal events quantified in terms of interval (top panel) and duration (second panel) ($n = 15$ for NEC; $n = 17$ for pilocarpine-treated); PC ictal-like events quantified in terms of frequency (third panel) and duration (bottom panel) in 4AP condition (black) and THDOC condition (white) ($n = 12$ for NEC; $n = 8$ for pilocarpine-treated; * $p < 0.05$; ** $p < 0.01$).

Fig. 5.

Effect of THDOC on 4AP-induced epileptiform activity in EC. (A) Interictal (asterisks) and ictal discharges recorded from EC of non-epileptic control (NEC) slices in 4AP and after bath application of THDOC. (B) Interictal (asterisks) and ictal discharges recorded from EC of epileptic (NEC) slices before and after (right) bath application of THDOC. (C) EC interictal events quantified in terms of frequency (top panel) and duration (second panel) ($n = 9$ for NEC; $n = 14$ for pilocarpine-treated); EC ictal-like events quantified in terms of frequency (third panel) and duration (bottom panel) in 4AP condition (black) and THDOC condition (white) ($n = 9$ for NEC; $n = 10$ for pilocarpine-treated; * $p < 0.05$; ** $p < 0.01$).

Fig. 6.

Effect of THDOC on HFOs associated with 4AP-induced interictal events. (A) Bar graphs quantify the effects of THDOC on the occurrence of (a) ripples and (b) fast ripples during interictal discharges in PC (NEC: $n = 15$, events = 4167; pilocarpine-treated: $n = 17$, events = 1215). (B) Bar graphs quantify the effects of THDOC on the occurrence of (a) ripples and (b) fast ripples coinciding with interictal discharges in EC (NEC: $n = 9$, events = 1209; pilocarpine-treated: $n = 14$, events = 1033; * $p < 0.05$; ** $p < 0.01$).

Fig. 7.

Effects of THDOC on HFOs underlying 4AP-induced ictal discharges. (A) Rates of ripple and fast ripple occurrence in the pre-ictal, ictal, and post-ictal phases of events recorded in (a) control and (b) epileptic slices. (NEC: $n = 12$, events = 72; pilocarpine-treated: $n = 8$, events = 54). (B) Rates of ripple and fast ripple occurrence in the pre-ictal, ictal, and post-ictal phases of events recorded in (a) control and (b) epileptic slices. (NEC: $n = 9$, events = 38; pilocarpine-treated: $n = 10$, events = 41; * $p < 0.05$; ** $p < 0.01$).

Summary of the effects induced by THDOC on interictal and ictal epileptiform discharges occurring in the PC and EC of NEC and pilocarpine-treated epileptic rats during 4AP application. Abbreviations are as follows: NC, no change; ↓, decrease; ↑, increase.

Table 1

	PC				EC			
	Interictal discharges		Ictal discharges		Interictal discharges		Ictal discharges	
	Frequency	Duration	Frequency	Duration	Frequency	Duration	Frequency	Duration
NEC	NC	↑	NC	a ↓	NC	NC	NC	b
Pilocarpine-treated	↓	↑	↓	c	NC	↑	↓	d

^aTHDOC abolished ictal events in 3/15 NEC slices (20.0%).

^bTHDOC abolished ictal events in 1/10 NEC slices (10.0%).

^cTHDOC abolished ictal events in 5/13 epileptic slices (38.5%).

^dTHDOC abolished ictal events in 4/14 slices of epileptic slices (28.6%).

Summary of the effects induced by THDOC on the HFOs associated to interictal and ictal epileptiform discharges occurring in the PC and EC of NEC and pilocarpine-treated epileptic rats during 4AP application. Abbreviations are as follows: NC, no change; ↓, decrease; ↑, increase.

Table 2

	PC				EC			
	Interictal discharges		Ictal discharges		Interictal discharges		Ictal discharges	
	Ripples	Fast ripples	Ripples	Fast ripples	Ripples	Fast ripples	Ripples	Fast ripples
NEC	NC	↓	NC	↓ <i>a</i>	NC	NC	NC	NC
Pilocarpine-treated		↑		↑ <i>c</i>	NC	↓ <i>d</i>	↓ <i>e</i>	↓ <i>f</i>

*a*THDOC increased ripples occurring during the pre- and post-ictal periods.
*b*THDOC increased fast ripples occurring during the pre-ictal period.
*c*THDOC increased ripple rates during the late phase of the ictal discharge.
*d*THDOC increased fast ripple rates during the late phase of the ictal discharge.
*e*THDOC increased ripple rates during the late phase of the ictal discharge.
*f*THDOC increased fast ripple rates during the late phase of the ictal discharge.