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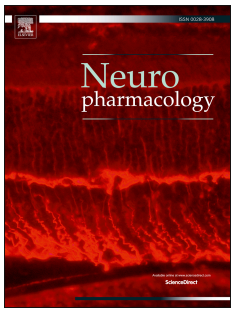

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ALLOPREGNANOLONE DECREASES INTERICTAL SPIKING AND FAST RIPPLES IN AN ANIMAL MODEL OF MESIAL TEMPORAL LOBE EPILEPSY

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ABSTRACT

The objective of this study was to characterize the impact of allopregnanolone, a neurosteroid that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors, on interictal spikes and high-frequency oscillations (ripples: 80-200 Hz, fast ripples: 250-500 Hz) in the pilocarpine model of mesial temporal lobe epilepsy. Seven out of 25 Sprague-Dawley rats experiencing 1 hour of pilocarpine-induced *status epilepticus* (SE) began treatment with allopregnanolone (9.6-12.8 mg/kg/day) on the following day. On day 4 after SE, video-depth EEG recordings from the hippocampal CA3 subfield and the entorhinal cortex were initiated and continued for 12 consecutive days. We found that 66.7% (12/18) of untreated animals exhibited seizures compared to 28.6% (2/7) of allopregnanolone-treated animals. Interictal spikes occurred less frequently in the CA3 subfield of allopregnanolone-treated rats ($n = 4$) than in untreated animals presenting ($n = 4$) or not presenting ($n = 4$) with spontaneous seizures ($p < 0.05$), and were less frequent in the entorhinal cortex compared to both untreated groups ($p < 0.05$). Finally, allopregnanolone-treated rats had significantly lower rates of interictal spikes with fast ripples (250-500 Hz) compared to untreated animals but only in CA3 ($p < 0.05$). Our findings show that allopregnanolone reduces the frequency of interictal spikes and fast ripples in CA3, a structure that plays an important role in ictogenesis and epileptogenesis. Neurosteroids may therefore influence pathological network activity leading to spontaneous seizures following pilocarpine-induced SE. Recordings after termination of allopregnanolone treatment will be however required to establish whether allopregnanolone exerts disease-modifying properties.

Keywords: neurosteroids; allopregnanolone; mesial temporal lobe epilepsy; pilocarpine; high-frequency oscillations.

1. INTRODUCTION

Allopregnanolone is a neurosteroid that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors. An endogenous compound, allopregnanolone is synthesized from ovarian progesterone in the periphery and *de novo* from cholesterol in glutamatergic principal neurons in the brain (Agís-Balboa et al., 2006; Mody, 2012; Reddy, 2010; Reddy and Rogawski, 2012). Since $GABA_A$ receptors are the main mediators of fast inhibitory neurotransmission (Avoli and Krnjević, 2016), alterations in allopregnanolone and related $GABA_A$ receptor active neurosteroids can have profound effects on neural network excitability. For instance, withdrawal of allopregnanolone at menstruation predisposes women with epilepsy to catamenial seizures (Reddy and Rogawski, 2009, 2012). Exogenously administered allopregnanolone protects against seizures induced by various convulsant stimuli in seizure models using non-epileptic animals, such as in the pentylenetetrazole, 6-Hz, pilocarpine, kainic acid and NMDA seizure tests (Kaminski et al., 2004; Kokate et al., 1996). In addition, allopregnanolone inhibits the behavioural seizure stage and afterdischarge duration of seizures provoked by electrical stimulation in the amygdala kindling epilepsy model (Lonsdale and Burnham, 2007; Reddy et al., 2012). There is also evidence that neurosteroids may have antiepileptogenic properties since the duration of the latent period in the pilocarpine model of mesial temporal lobe epilepsy (MTLE) is shortened when their synthesis is blocked (Biagini et al., 2006, 2009).

In *in vitro* brain slice preparations, $GABA_A$ receptor positive modulatory neurosteroids, including allopregnanolone, cause a concentration-dependent suppression of the epileptiform activity induced by the K^+ channel blocker 4-aminopyridine or the $GABA_A$ receptor antagonist picrotoxin (Herrington et al., 2014; Salazar et al., 2003). In addition, the related neurosteroid allotetrahydrodeoxycorticosterone has been shown to reduce the occurrence of high-frequency oscillations (HFOs, ripples: 80-200 Hz, fast ripples: 250-500 Hz) that are associated with 4-aminopyridine-induced epileptiform discharges in brain slices *in vitro* (Herrington et al., 2014,

2015). HFOs reflect the activity of dysfunctional neuronal networks (Jefferys et al., 2012) and are considered to be biomarkers of epileptogenesis and ictogenesis in animal models of MTLE (Bragin et al., 2004; Lévesque et al., 2011, 2012) and in epileptic patients (Jacobs et al., 2009; Staba et al., 2002).

To date, no study has addressed the impact of continuous, systemic administration of a $GABA_A$ receptor positive modulatory neurosteroid on spontaneous seizures in the pilocarpine model of MTLE. Moreover, it is unclear whether any antiseizure effects of neurosteroids obtained in this situation are accompanied by changes in interictal spike or HFO occurrence. Therefore, we used depth brain EEG recordings from the hippocampal CA3 subfield and the entorhinal cortex (EC) to establish the effects induced by continuous treatment with allopregnanolone in Sprague-Dawley rats following pilocarpine-induced SE.

2. MATERIAL AND METHODS

2.2. Ethical approval - All procedures were approved by the Canadian Council of Animal Care and the McGill University Animal Care Committee. All efforts were made to minimize the number of animals and their suffering.

2.3. Pilocarpine treatment - Male Sprague-Dawley rats (150-200 g, 45 to 50 days old) were purchased from Charles River Laboratories (St-Constant, Qc, Canada) and allowed to habituate for 72 hours after delivery before pilocarpine treatment. On the day of SE induction, they received scopolamine methylnitrate (1 mg/kg i.p.; Sigma-Aldrich, Qc, Canada). Thirty minutes later, they were injected with a single dose of pilocarpine hydrochloride (380 mg/kg, i.p.; Sigma-Aldrich, Qc, Canada) (Lévesque et al., 2012; Salami et al., 2014). Their behavior was scored according to the Racine scale (Racine, 1972). SE was defined as continuous stage 5 seizure activity. SE was terminated after 1 h by injecting diazepam (5 mg/kg, s.c.; CDMV, Qc, Canada)

and ketamine (50 mg/kg, s.c.; CDMV, Qc, Canada) (Martin and Kapur, 2008). Animals that did not experience SE were excluded from further analysis.

2.4. Treatment with allopregnanolone - On the day following *status epilepticus* (SE), a 2 ml ALZET osmotic minipump (DURECT Corporation, CA, USA) calibrated to deliver 5.0 µl/hr was subcutaneously implanted on the dorsal side under light isoflurane anesthesia (2% in 100% O_2) in order to deliver allopregnanolone solution for 12 consecutive days (n = 7 animals). The entire surgical procedure lasted less than 5 minutes. To prepare the treatment solution, 2 g of sulfobutyl ether-β-cyclodextrin (SBEBCD; Captisol, Ligand Pharmaceuticals, CA, USA) was dissolved in 5 ml of water and 0.08 g of allopregnanolone was added to obtain a concentration of 16 µg/ml. Therefore, the dose rate (k_0) of allopregnanolone for rats weighing between 150 and 200 g would be from 9.6 mg/kg/day to 12.8 mg/kg/day. The clearance (CL) of allopregnanolone in rats has been determined to be 4.8 l/h/kg (Martinez Botella et al., 2015). The expected steady-state blood concentration can be estimated as k_0/CL, which is 83-110 ng/ml. Immediately following implantation of the pump, animals were injected with a 15 mg/kg loading dose of allopregnanolone to avoid a delay in achieving the steady-state level. Some untreated animals were treated with saline (n = 11) and others received no treatment (n = 7). Since seizure rates were not significantly different between animals treated with saline and animals that received no treatment, data obtained from these two groups were pooled together and compared with those from allopregnanolone-treated animals.

2.5. Stereotaxic surgery - On the third day after SE, rats were anaesthetized with isoflurane (3%) in 100% O_2 and fixed in a stereotaxic frame. An incision was made in the skin and four stainless steel screws (2.4 mm length) were fixed to the skull. Three small holes were also drilled to allow the implantation of bipolar electrodes (20-30 kΩ; distance between exposed tips:

500 µm). Electrodes were implanted in the CA3 subfield of the hippocampus (AP: -4.4, ML: ± 4.2, DV: - 4.3) and the EC (AP: - 6.6, ML: ± 4, DV: -8.8) since these regions are often seizure onset zones in this animal model of MTLE (Bortel et al., 2010; Lévesque et al., 2011, 2012; Salami et al., 2014). Screws and electrodes were fastened to the skull with dental cement. A fifth bipolar electrode was placed under the frontal bone, after the removal of the insulating material, and used as reference. After surgery, animals received topic application of chloramphenicol (Erfa, Qc, Canada) and lidocaine (5%; Odan, Qc, Canada) and were injected with ketoprofen (5mg/kg s.c.; Merail, Qc, Canada), buprenorphine (0.01-0.05 mg/kg s.c., CDMV, Qc, Canada) and 2 ml of 0.9% saline (s.c.).

2.6. EEG recordings - After surgery, rats were housed individually in custom-made Plexiglas boxes (30 x 30 x 40 cm). The recording electrodes were connected to a multichannel cable and electrical swivel (Commutator SL 18C, HRS Scientific, Qc, Canada) and continuous EEG recordings were collected for 12 days. EEG signals were amplified via an interface kit (Mobile 36ch LTM ProAmp, Stellate, Qc, Canada); they were low-pass filtered at 500 Hz and sampled at 2 kHz per channel. EEG recordings were performed using monitoring software (Harmonie, Stellate, Qc, Canada). Throughout the recordings, animals were placed under controlled conditions (22 ± 2 ˚C, 12 h light/dark schedule) and were provided with food and water *ad libitum.*

2.7. Seizure analysis - All EEG and video recordings were reviewed manually in order to detect seizures. The occurrence of the first spontaneous seizure (convulsive or non-convulsive) after SE marked the end of the latent period.

2.8. Interictal spike and HFO analysis - We compared rates of interictal spikes and rates of HFOs from the untreated and the allopregnanolone-treated group. For each animal selected for

analysis, one epoch of 10 min was extracted for each day of recording. Only epochs of non-REM sleep were used for analysis because of the low rates of movement artefacts and because HFOs are more prominent during this sleep stage (Bagshaw et al., 2009). Extracted epochs were exported to Matlab 7.11.0 (The Mathworks, MA, USA) using custom-built routines and analyzed off-line. Interictal spikes were detected based on threshold crossings (mean and standard deviation (SD)), calculated over the entire period for the 10-min epoch. Events above 4 SD were considered as interictal spikes. Every detected event was analysed visually and false positives caused by movement artefacts were removed. Rates of interictal spikes (number of events/s) were then calculated for each region and averaged in order to obtain a single value per day per animal.

The same epochs used to detect interictal spikes were used for the analysis of HFOs. The algorithm employed to detect these events was previously published (Lévesque et al., 2011; Salami et al., 2014) and will be briefly described here. A custom-built algorithm in Matlab 7.11.0 (The Mathworks, MA, USA) was used to filter raw EEG recordings in the 80–200 Hz and in the 250–500 Hz frequency range. Filtered EEGs from each region were then normalized using their own average calculated over the 10 min epoch. To be considered as an HFO candidate, oscillatory events in each frequency band had to show at least 4 consecutive cycles having amplitude of 3 SD above the mean. HFOs were considered as co-occurring with a spike if they occurred within a time window of 300 ms from the peak of an interictal spike. Overlapping events, which may be caused by the filtering of sharp spikes (Bénar et al., 2010), were excluded from the analysis. Visual validation was also performed to eliminate false positives created by movement artifacts. In order to reduce the variability of HFOs due to interictal spike occurrence, we calculated the ratio of interictal spikes with ripples to interictal spikes as well as the ratio of interictal spikes with fast ripples to interictal spikes. These ratios indicated whether interictal spikes with ripples or fast ripples changed in CA3 or EC in untreated animals or in allopregnanolone-treated animals.

2.9. Wavelet analysis – HFOs were visible on a color spectrogram that was obtained using a wavelet transform with custom-built functions in Matlab 7.11.0 (The Mathworks, MA, USA). This allowed us to examine changes in power in the time frequency domain. The wavelet coefficients of the signal were calculated, spanned over 1-500 Hz with steps of 0.01 Hz and a time resolution of 0.5 ms.

2.10. Statistical analysis – Results throughout this study are expressed as mean (± standard error of mean). Wilcoxon rank-sum and Kruskall-Wallis tests followed by Tukey post-hoc tests were used in order to compare values obtained in each group, since values were not normally distributed according to the Kolmogorov–Smirnov test. Fisher's exact test was employed to determine if the difference between the proportion of animals with seizures and without seizures was significant. The level of significance was set at $p < 0.05$.

3. RESULTS

3.1. Seizures – As previously reported in the pilocarpine model of MTLE (Bortel et al., 2010; Lévesque et al., 2011, 2012; Salami et al., 2014), animals showed spontaneous recurrent behavioural and electrographic seizures after SE. Seizures were observed in both untreated and allopregnanolone-treated animals. Electrographic seizures always occurred in both CA3 and EC, and were characterised by low amplitude fast activity (10-20 Hz) at onset (Fig 1A, B, insets), followed by low-frequency high amplitude spikes. We considered as epileptic only animals that exhibited at least two seizures that occurred more than 24 h apart (Fisher, 2015). In the untreated group, 12 rats out of 18 (66.7%) were classified as epileptic compared to 2 rats out of 7 (28.6%) in the allopregnanolone-treated group (Fisher's exact test: $p = 0.18$). Since only a small number of allopregnanonolone-treated animals were epileptic, we could not perform any

further statistical comparisons on seizures between the untreated group and the allopregnanonolone-treated group.

Figure 1 approx. here

3.2. Interictal spikes - Interictal spike activity was analysed in recordings obtained from untreated animals with (n = 4) or without (n = 4) spontaneous seizures and from allopregnanolone-treated animals that did not present with spontaneous seizure activity (n = 4). We sought to determine if the absence of spontaneous seizures in some allopregnanolone-treated animals was associated with a reduced frequency of interictal spikes. We could not perform any statistical analyses on the epileptic allopregnanolone-treated animals due to the small number of animals in this group (n = 2).

Interictal spikes were recorded from the CA3 and EC electrodes in the three groups analyzed (Fig 2A-C). We observed a significant difference in the interictal spike rates among the three groups in both CA3 (df = 2, chi-square = 26.8 $p < 0.01$) and EC (df = 2, chi-square = 20.8, $p < 0.01$). Specifically, post-hoc tests revealed that non-epileptic allopregnanolone-treated animals had significantly lower rates of interictal spikes compared to both (epileptic and non-epileptic) untreated animals ($p < 0.05$) in CA3 (Fig 2D). Non-epileptic untreated animals also showed significantly lower rates of interictal spikes in CA3 compared to epileptic untreated animals ($p < 0.05$) (Fig. 2D). Finally, in the EC, rates of interictal spikes were significantly lower in non-epileptic allopregnanolone-treated animals compared to epileptic untreated animals ($p < 0.05$) (Fig 2D).

Figure 2 approx. here

3.4. Interictal spikes with high-frequency oscillations - Interictal spikes in all groups could co-occur in association with ripples (80-200 Hz) or fast ripples (250-500 Hz), as previously reported in epileptic patients and in animal models (Bragin et al., 2007; Jacobs et al., 2009).

Detected ripples (Fig 3A) and fast ripples (Fig 4A) were visible with the use of a wavelet time-frequency analysis. In order to exclude any effect on HFOs caused by the variability in interictal spike occurrence, we calculated the ratio of interictal spikes with HFOs to the total number of interictal spikes in CA3 and EC in the three groups. This analysis revealed that the ratio of interictal spikes with ripples to interictal spikes did not differ among groups in both CA3 and EC (Fig 3B).

Figure 3 approx. here

However, we observed a significant effect of allopregnanolone on interictal spikes with fast ripples in CA3 ($df = 2$, chi-square = 17.9, $p < 0.001$). Specifically, in this limbic area, the proportion of interictal spikes with fast ripples to interictal spikes was significantly lower in the allopregnanolone-treated group compared to epileptic and non-epileptic untreated animals ($p < 0.05$) (Fig 4B). In EC, we also observed a significant effect ($df = 2$, chi-square = 17.7, $p < 0.001$); post-hoc analyses revealed that the proportion of interictal spikes with fast ripples to interictal spikes in non-epileptic untreated animals was significantly lower than in epileptic untreated animals and in non-epileptic allopregnanolone-treated animals ($p < 0.05$) (Fig 4B). Altogether, these results indicate that allopregnanolone modulates the occurrence of interictal spikes associated with fast ripples but not those associated with ripples in the CA3 region of the hippocampus.

Figure 4 approx. here

4. DISCUSSION

The main observation in this study is that allopregnanolone treatment is associated with a marked reduction in interictal spikes. When comparing non-epileptic untreated animals, epileptic untreated animals and non-epileptic allopregnanolone-treated animals, we found that in CA3, interictal spikes occurred less frequently in non-epileptic allopregnanolone-treated animals than in untreated animals presenting or not presenting seizures. In EC, interictal spikes also occurred less frequently in non-epileptic allopregnanolone-treated animals than in both groups of

untreated animals. Moreover, in CA3 but not in EC, allopregnanolone treatment was associated with a significant decrease in the frequency of interictal spikes with fast ripples compared with the frequency in epileptic and non-epileptic untreated animals. Therefore, our findings indicate that allopregnanolone inhibits the occurrence of interictal activity when administered continuously in the early period (first 2 weeks) after pilocarpine-induced SE. This effect is accompanied by low fast ripple rates in the CA3 region of the hippocampus, which is known to play an important role in ictogenesis and epileptogenesis in this model.

4.1. Spontaneous seizures

Previous studies have shown that the decreased synthesis of allopregnanolone can accelerate the appearance of spontaneous seizures after pilocarpine-induced SE (Biagini et al., 2006). Although evaluation of the antiepileptogenic properties of allopregnanolone was not an objective of this study, our findings show that 28.6% of allopregnanolone-treated animals became epileptic compared to 66.7% of untreated animals. Therefore, our results are consistent with the possibility that allopregnanolone has antiepileptogenic actions. However, since our recordings were conducted during the continuous delivery of allopregnanolone, the effects observed could be due to the pharmacological actions of the neurosteroid and do not necessarily imply disease modification. Recordings following wash out of allopregnanolone will be indeed required to assess antiepileptogenesis.

The effect of neurosteroids is presumably due to the action of allopregnanolone on $GABA_A$ receptor signaling (Salazar et al., 2003). Several studies have shown that the development of spontaneous seizures in rodents that have experienced pilocarpine-induced SE depends, at least in part, on GABAergic cell loss (de Guzman et al., 2006; Houser and Esclapez, 1996; Peng et al., 2013; Soukupová et al., 2014). As a positive modulator of $GABA_A$ receptors, allopregnanolone may functionally reverse the GABAergic deficit. In this respect, it should be noted that allopregnanolone is not effective in preventing seizures that are not

suppressed by agents that enhance $GABA_A$ receptor signaling. For instance, allopregnanolone does not exhibit protective activity in the maximal electroshock test, in which other modulators of $GABA_A$ receptors, such as benzodiazepines, are also ineffective (Reddy and Rogawski, 2012; Rogawski et al., 2013). In addition, it has been reported that allopregnanolone does not control strychnine-induced seizures (Belelli et al., 1989).

4.2. Interictal spikes and high-frequency oscillations (80-500 Hz)

We found that in the CA3 subfield, interictal spike rates were significantly reduced in allopregnanolone-treated animals compared to epileptic and non-epileptic untreated animals. *In vitro*, allopregnanolone also inhibits interictal discharges in the 4-aminopyridine model (Salazar et al., 2003) while in women with partial epilepsy, continuous intravenous administration of progesterone, a precursor for allopregnanolone, is effective in reducing interictal spike occurrence (Bäckström et al., 1984). Therefore, our results indicate that continuous exposure for days to allopregnanolone causes a persistent reduction in brain excitability reflected in a reduced frequency of interictal spiking. It is well recognized that acute allopregnanolone administration protects against seizures and can terminate SE (Reddy and Rogawski, 2012). We now show for the first time confirm that chronic exposure to allopregnanolone persistently suppresses interictal epileptiform activity.

We also discovered that allopregnanolone treatment is associated with a decrease in HFOs, which are believed to be biomarkers of pathological neuronal network hyperexcitability (Jefferys et al., 2012). It was previously reported that low rates of HFOs occur following the application of neurosteroids in the *in vitro* 4-aminopyridine model (Herrington et al., 2015; Shiri et al., 2015). However, to our knowledge, the present study represents the first demonstration that neurosteroids have modulatory effects on HFOs in an *in vivo* chronic model of MTLE. In CA3, we observed that the ratio of interictal spikes with fast ripples to interictal spikes was smaller in allopregnanolone-treated animals than in epileptic and non-epileptic untreated

animals. This finding suggests that allopregnanolone treatment inhibits fast ripples, which are believed to be generated from the uncontrolled in phase or out of phase firing of principal cells due to a collapse of perisomatic inhibition (Bragin et al., 2011; Foffani et al., 2007; Gulyás and Freund, 2015; Ibarz et al., 2010).

During the latent period prior to the onset of seizures, the occurrence of HFOs in temporal lobe regions predicts the later occurrence of spontaneous seizures (Bragin et al., 2004). In contrast, during the period of established spontaneous seizures, HFOs indicate time periods of high susceptibility to seizures (Lévesque et al., 2011). The properties of HFOs also change over time, since they can evolve from short to prolonged events within two weeks after status epilepticus (Jones et al., 2015). The evolution of fast ripples has been proposed as a marker of the progressive increase in excitatory drive that reflects the pathological and epileptogenic reorganization of neuronal networks in the limbic system leading to the generation of spontaneous seizures (van Diessen et al., 2013).

Several studies have identified a relation between fast ripples and epileptogenesis in kainic acid and pilocarpine post-SE epilepsy models (Bragin et al., 2004, 2011; Jones et al., 2015; Lévesque et al., 2011; Salami et al., 2014). Hence, our findings suggest that neurosteroids may control the plastic processes that occur during epileptogenesis since we have observed a significant effect on fast ripples in CA3. It must however be acknowledged that all studies to date assessing the antiepileptogenic role played by neurosteroids have been indirect and not definitive; more direct studies are clearly needed.

5. CONCLUSIONS

Our experiments show that allopregnanolone is effective in reducing the occurrence of interictal spiking and HFOs in the pilocarpine model of MTLE. Allopregnanolone is a positive modulator of $GABA_A$ receptor signalling, an action that likely accounts for the reduction in interictal events,

which reflect neuronal network hyperexcitability. Interestingly, the effects of allopregnanolone identified here are similar to those obtained in previous studies in which antiseizure drugs such as levetiracetam and lacosamide were used in the pilocarpine model of MTLE (Behr et al., 2015; Lévesque et al., 2014), confirming the potential of $GABA_A$ receptor modulating neurosteroids in the treatment of epilepsy in humans. Indeed, there is clinical evidence that the allopregnanolone analog ganaxolone is effective in the treatment of focal epilepsies (Reddy and Rogawski, 2012) but to date no neurosteroid based epilepsy treatment has been approved for human use. Our results support the promise of such agents and add to the evidence that neurosteroids, when administered early after SE, can suppress fast ripples, which are biomarkers of pathological network activity in epilepsy.

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DECLARATION OF INTEREST

The authors declare no conflicts of interest.

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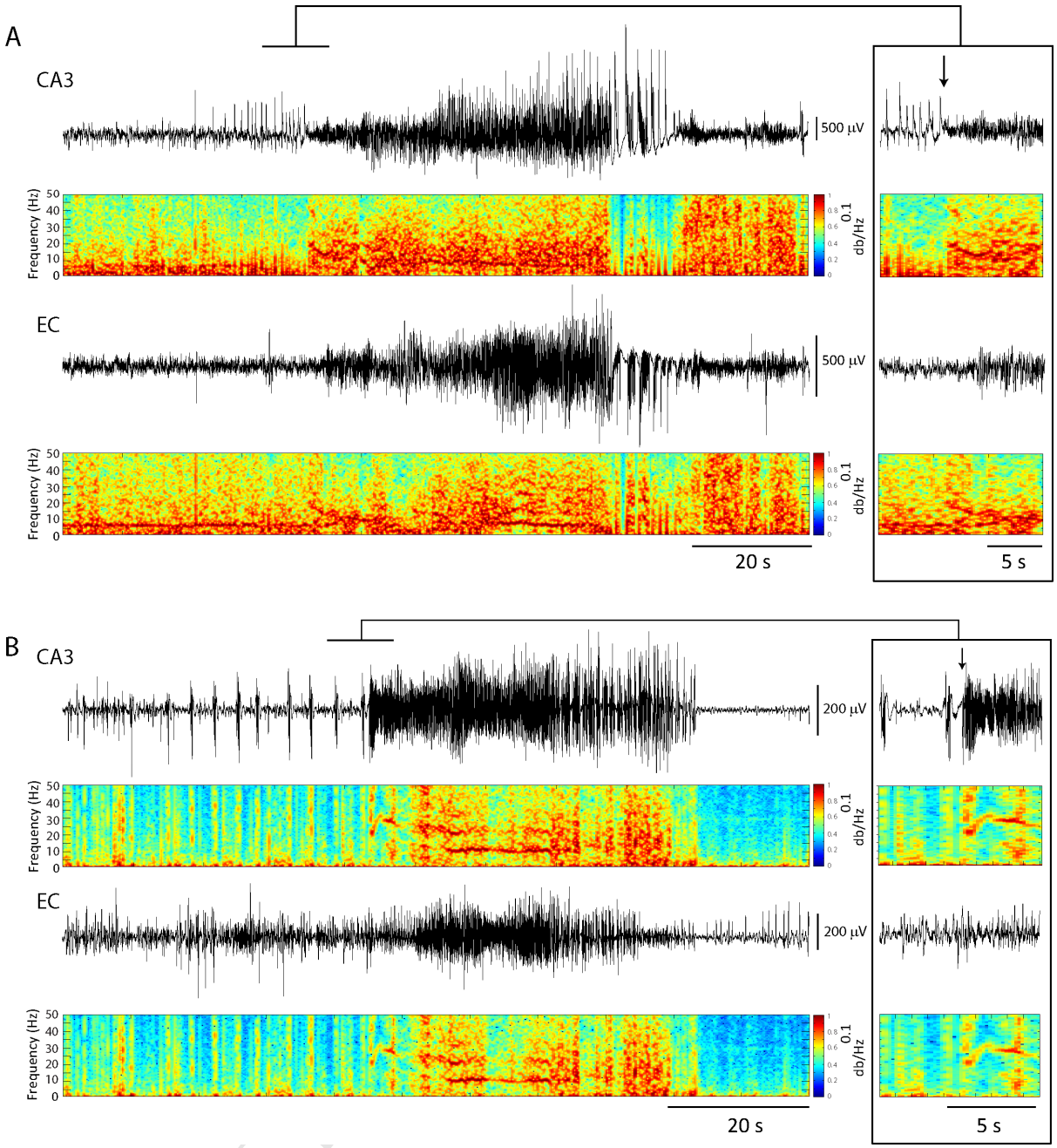
FIGURE LEGENDS

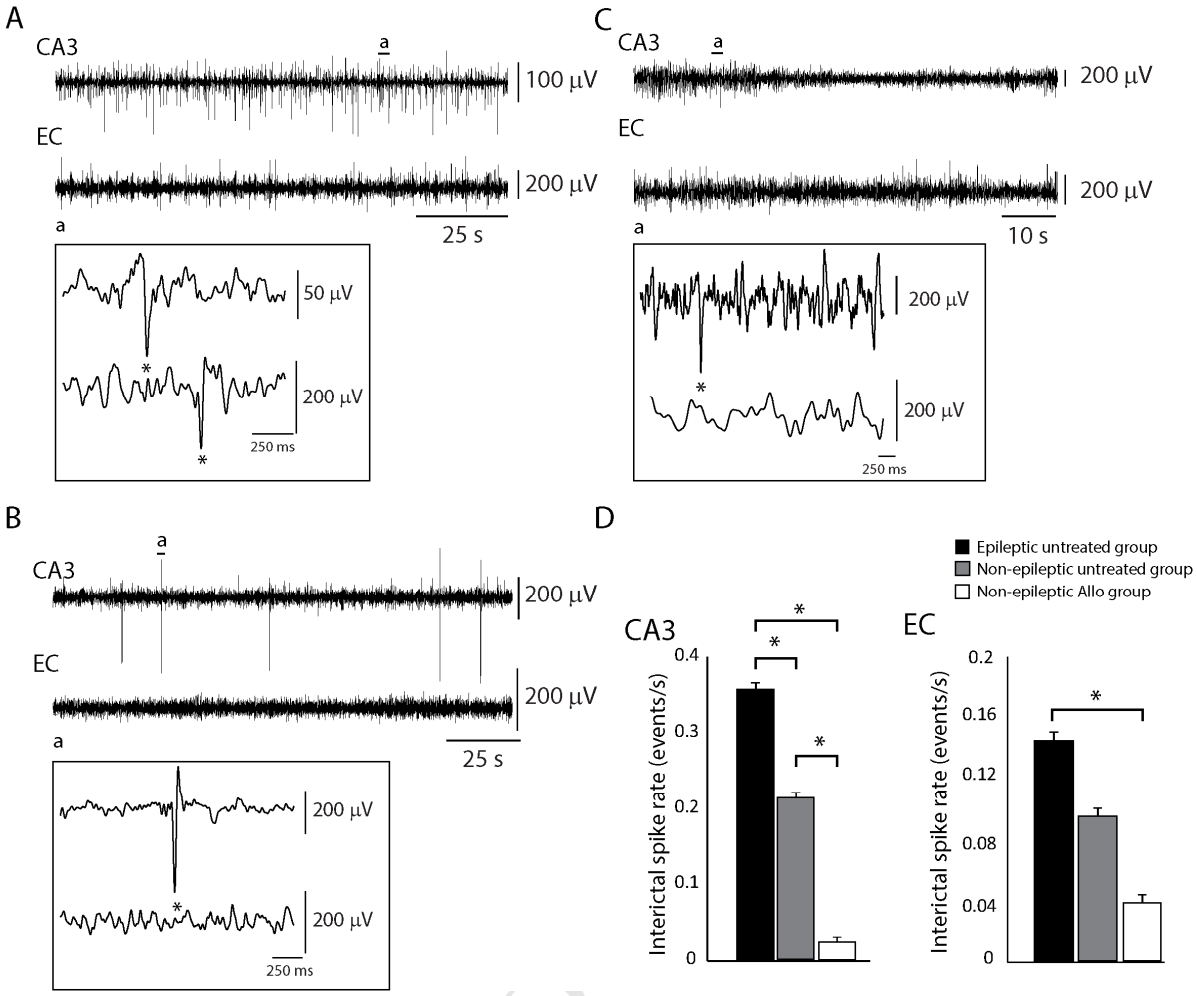
Figure 1 – Electrographic seizures in epileptic untreated and epileptic allopregnanolone-treated animals. A: Representative example of a seizure recorded in an untreated animal. **B:** Representative example of a seizure recorded in an allopregnanolone-treated animal. Note the high amplitude low frequency spikes that are similar to those recorded during seizures in epileptic untreated animals. Field potentials shown in this figure were analysed using the spectrogram method with discrete short-time Fourier transform and data sets were separated into 1 s intervals to which a Hamming window was applied. To enhance the detection of oscillations, a gamma correction with a factor of 0.1 was applied to the spectrogram to improve their contrast to random noise.

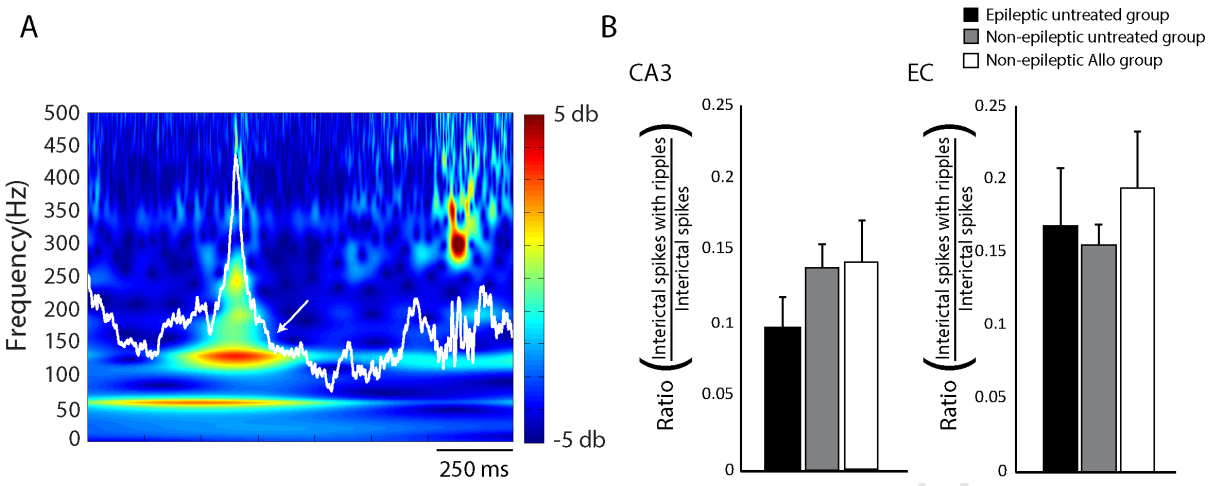
Figure 2 – Interictal spike activity patterns. A: Representative example of interictal spike activity in an epileptic untreated animal. The inset shows interictal spikes (asterisks) occurring in both CA3 and EC. **B:** Representative example of interictal spike activity in a non-epileptic untreated animal. The inset shows an interictal spike (asterisk) occurring in CA3; no spike occurred in EC. **C:** Representative example of interictal spike activity in a non-epileptic allopregnanolone-treated animal. The inset shows an interictal spike (asterisk) occurring in CA3. **D:** Bar graph showing the interictal spike rates in the epileptic untreated group, the non-epileptic untreated group and the non-epileptic allopregnanolone-treated group. In CA3, non-epileptic untreated animals showed significantly lower interictal spike rates compared to epileptic untreated animals. Non-epileptic allopregnanolone-treated animals showed significantly lower interictal spike rates compared to epileptic and non-epileptic untreated animals. In EC, interictal spike rates were significantly lower in non-epileptic allopregnanolone-treated animals compared to epileptic untreated animals. (* $p < 0.05$). Allo = allopregnanolone.

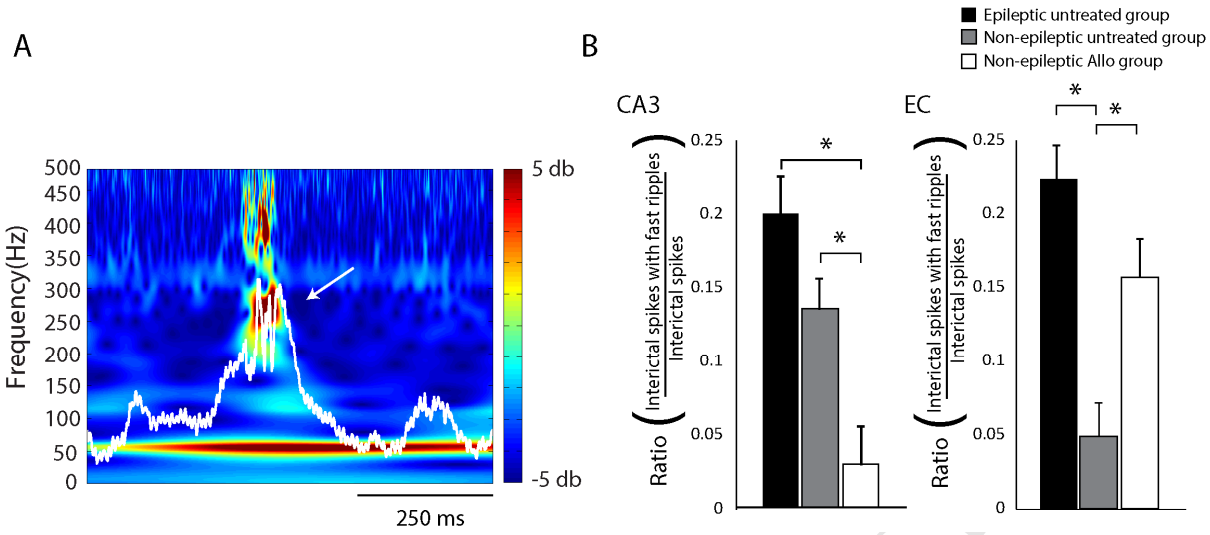
Figure 3 – Interictal spikes with ripples (80-200 Hz) – A: Interictal spike (white trace) from an epileptic untreated animal with its corresponding wavelet analysis. Note the occurrence of a ripple (arrow) that is characterized by frequencies between 100 and 150 Hz in the wavelet time-frequency analysis. **B:** Bar graphs showing the average ratio of interictal spikes with ripples to interictal spikes. In both CA3 and EC, no significant differences were observed among the three groups.

Figure 4 – Interictal spikes with fast ripples (250-500 Hz) – A: Interictal spike (white trace) from the same animal as in figure 3A. Note the occurrence of a fast ripple (arrow) that is characterized by frequencies between 250 and 300 Hz. **B:** Bar graphs showing in CA3 and EC the average ratio of interictal spikes with fast ripples to interictal spikes. In CA3, this ratio was significantly lower in non-epileptic allopregnanolone-treated animals compared to epileptic and non-epileptic untreated animals. In EC, non-epileptic untreated animals showed significantly lower rates compared to epileptic untreated animals and non-epileptic allopregnanolone-treated animals (*$p < 0.05$).









Allopregnanolone was administered continuously to pilocarpine-treated animals

Interictal spikes were less frequent in the CA3 subfield and entorhinal cortex

Fast ripples (250-500 Hz) were less frequent in the CA3 subfield

Neurosteroids may prevent the development of pathological activity