High frequency oscillations can pinpoint seizures progressing to status epilepticus

Pariya Salami, Maxime Lévesque, and Massimo Avoli*

^aMontreal Neurological Institute, McGill University, Montréal H3A 2B4 QC, Canada

^bDepartment of Neurology & Neurosurgery, McGill University, Montréal H3A 2B4 QC, Canada

^cDepartment of Physiology, McGill University, Montréal H3A 2B4 QC, Canada

Abstract

Status epilepticus (SE) is defined as a seizure lasting more than 5 min or a period of recurrent seizures without recovery between them. SE is a serious emergency condition that requires immediate intervention; therefore, identifying SE electrophysiological markers may translate in prompt care to stop it. Here, we analyzed the EEG signals recorded from the CA3 region of the hippocampus and the entorhinal cortex in rats that responded to systemic administration of 4-aminopyridine (4AP) by generating either isolated seizures or seizures progressing to SE. We found that high frequency oscillations (HFOs) – which can be categorized as ripples (80–200 Hz) and fast ripples (250–500 Hz) – had different patterns of occurrence in the two groups ($n = 5$ for each group). Specifically, fast ripples in CA3 and entorhinal cortex of the SE group occurred at higher rates than ripples, both during the ictal and post-ictal periods when compared to the HFOs recorded from the isolated seizure group. Our data reveal that different patterns of HFO occurrence can pinpoint seizures progressing to SE, thus suggesting the involvement of different neuronal networks at the termination of seizure discharges.

Keywords

Status epilepticus; 4-Aminopyridine; High frequency oscillations; Entorhinal cortex; Hippocampus

1. Introduction

Status epilepticus (SE) is defined as a period of prolonged seizure activity lasting more than 5 min or as a series of recurrent seizures with no regain of normal function between them (Brophy et al., 2012). SE is considered as a serious neurological emergency and immediate care to stop seizure activity is required (Brophy et al., 2012; Walker, 2005). Understanding the mechanisms that sustain seizures during SE and identifying the electrographic features pinpointing this clinical condition (*i.e.*, the characteristics of those seizures that will progress

*Corresponding author at: Montreal Neurological Institute, 3801 University Street, Montréal, PQ H3A 2B4, Canada. massimo.avoli@mcgill.ca (M. Avoli).

Conflicts of interest

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into SE) should provide helpful information for the development of early diagnosis and, hopefully, of new therapeutic treatments aimed to stop it.

High frequency oscillations (HFOs), *i.e.*, ripples (80–200 Hz) and fast ripples (250–500 Hz) have shown to be highly correlated with seizures in patients suffering from focal epileptic disorders such as temporal lobe epilepsy as well as in animal models mimicking this neurological condition (Bragin et al., 1999; Jirsch et al., 2006; Lévesque et al., 2012). Pathological HFOs are most often recorded from brain regions involved in seizure generation (Jacobs et al., 2010; Lévesque et al., 2012; Zijlmans et al., 2009) and their generation may be contributed by several mechanisms including excitatory and inhibitory signaling, out-of-phase firing of neuronal clusters, and interneuronal coupling through gap junctions (see for reviews Buzsáki and da Silva, 2012; Jefferys et al., 2012). In particular, it has been proposed that ripples may mirror synchronous IPSPs generated by principal cells in response to inhibitory interneuron firing while fast ripples are proposed to reflect abnormal synchronous firing of principal cells, thus being presumably independent of inhibitory neuro-transmission (Bragin et al., 1999, 2011; Dzhala and Staley, 2004; Foffani et al., 2007; Ibarz et al., 2010; Jefferys et al., 2012). However, a recent study suggests that HFOs should be distinguished based on their underlying mechanisms and not their frequency content (Alvarado-Rojas et al., 2015). Further studies are therefore required to elucidate the mechanisms underlying HFOs.

Seizure activity is induced by 4-aminopyridine (4AP) in animals both *in vitro* (Avoli et al., 1996; Klueva et al., 2003; Uva et al., 2015) and *in vivo* (Fragoso-Veloz et al., 1990; Lévesque et al., 2013; Mihály et al., 1990; Salami et al., 2015) preparations as well as in humans following accidental overdose (Schwam, 2011). In addition, we have recently reported that HFOs can be recorded during seizures that are induced in rats by the systemic administration of 4AP (Salami et al., 2015). Therefore, we extended here these experiments to investigate the differences in HFO occurrence between animals that generated isolated seizures and those in which seizures progressed to SE. Specifically, we postulated that different neuronal networks may be involved in the termination of seizure activity in these two animal groups and we hypothesized that HFO characteristics should help in identifying animals generating isolated seizures from those progressing to SE.

2. Materials and methods

2.1. Animal housing

Adult male Sprague–Dawley rats (250–300 g) were obtained from Charles River (St-Constant, Qc, Canada), and were let habituate for 72 h after delivery before the implantation of depth EEG recording electrodes. These animals were housed in controlled conditions, at 22 ($\pm$ 2) °C and under a 12 h light/12 h dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) with food and water *ad libitum*. All experimental procedures were approved by the Canadian Council of Animal Care and all efforts were made to minimize suffering and the number of animals used.

2.2. Surgery for the implantation of electrodes

A total of 13 animals underwent surgery for the implantation of depth EEG recording electrodes before 4AP treatment. Before surgery, rats received topical Lidocaine (5%; Odan, Canada) in the areas that underwent surgical intervention. They were then anesthetized with isoflurane (3%) in 100% O₂ and positioned in a stereotaxic frame. An incision was then made in the skin to expose the skull plate, from bregma to lambda. Four stainless steel screws (2.4 mm length) were fixed to the skull and 3 small holes were drilled to allow the implantation of bipolar recording electrodes (20–30 k Ω ; 5–10 mm length; distance between exposed tips: 500 μ m) (MS303/2-B/spc, Plastics One, VA, USA). Electrodes were implanted in all animals in the CA3 region of the right hippocampus (AP: –4.3, ML: +4, DV: –7.8) and in the right entorhinal cortex (EC) (AP: –8.6, ML: +5.2, DV: –6.8) (Paxinos and Watson, 1998). A third bipolar electrode was placed under the frontal bone, after the removal of insulating material, and it was used as reference. During surgery, animals received a preventive antibiotic therapy (Enrofloxacin, 10 mg/kg, s.c.). After surgery, rats were injected with Ketoprofen (5 mg/kg, s.c. Merial, Canada), Buprenorphine (0.01–0.05 mg/kg, s.c., repeated every 12 h; CDMV, Canada) and 2 ml of 0.9% sterile saline (s.c.) repeated every 12 h if necessary.

2.3. EEG recording

Two days after surgery, rats were placed in custom-made Plexiglas boxes (30 $\times$ 20 $\times$ 40 cm) and provided with food and water *ad libitum*. Electrodes were then connected to multichannel cables and swivels. EEGs were amplified using an interface kit (Mobile 36 chITMPro Amp, Stellate), low-pass filtered at 500 Hz and sampled at 2 kHz per channel. On the day of injection, animals were given 4AP (4–5 mg/kg, i.p.) in order to induce acute seizures (Lévesque et al., 2013; Salami et al., 2015). EEG-video monitoring was performed using the Stellate system for at least 30 min before and 4 h following the injection of 4AP (Lévesque et al., 2013).

2.4. Seizure detection and classification

EEG recordings were visually analyzed for the detection of seizures. We identified in each animal the first seizure that occurred after 4AP injection and determined whether it remained isolated or it progressed to a condition characterized by repeated seizures thus replicating the SE condition. We then extracted time periods corresponding to 500 s before the onset and to 300 s after the end of each seizure. A time period of 300 s was selected in order to avoid the onset of a second seizure in the SE group. On average, the first seizure was followed by a second seizure 1012 ($\pm$ 398) s after. Data were exported to Matlab 7.11.0 (R2010b) (Mathworks, Natick, MA, USA) and were analyzed offline using custom-built routines.

2.5. High-frequency oscillation analysis

In order to study the temporal evolution of HFOs over time, rates of HFOs (number of HFOs per bin) were first calculated for each seizure. The distribution of ripples and fast ripples during the ictal and post-ictal periods was then averaged for both groups. The ictal period was normalized into a 100 bins to account for differences in seizure duration and for easier

comparison. The average rate of ripples and fast ripples was normalized according to the duration of the seizure to account for differences in seizure duration.

In order to detect HFOs, raw EEG recordings were first band-pass filtered in the 80–200 Hz and in the 250–500 Hz frequency range using a finite impulse response filter; zero-phase digital filtering was used to avoid phase distortion. Filtered EEGs from each region were then normalized using a 10 s reference period selected from 510 s to 500 s before the onset of the seizure. 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 SD above the mean of the reference period. 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. Ripples and fast ripples occurring at the same time (overlapping events) were removed from analysis to avoid the effect of sharp events (Bénar et al., 2010; Lévesque et al., 2012; Salami et al., 2012).

2.6. Statistical analysis

In order to compare the rates of occurrence of ripples and fast ripples in each region during the pre-ictal, ictal and post-ictal periods, we arbitrarily divided each period in three equal parts. We then compared the rate of occurrence of ripples and fast ripples using Wilcoxon signed-rank tests followed by Bonferroni–Holm corrections to correct for multiple comparisons. By using this procedure, we determined if ripples or fast ripples predominated at different time periods during the pre-ictal, ictal, and post-ictal periods. Kruskal–Wallis tests were used to compare the rate of occurrence of spikes during the pre-ictal period between regions and between groups. The level of significance was set to $p < 0.05$.

3. Results

Based on the EEG and the behavior of animals, we classified them in two groups. The first isolated seizure group, included 7 rats in which 4AP induced a single seizure, followed by a return to normal EEG activity and normal behavior characterized by exploration and grooming. The second group, the SE group, included 6 animals that showed multiple convulsive seizures associated to an abnormal behavior; these animals lied on their side during the entire recording period. These rats showed on average $2.4 (\pm 0.5)$ seizures on the EEG. On average, they died $42.5 (\pm 13.4)$ min after the onset of the first seizure. In both groups, we were unable to classify the first seizure into a low-voltage fast onset or a hypersynchronous onset pattern (*cf.*, Lévesque et al., 2013; Salami et al., 2015). Examples of the first seizure occurring in rats of the isolated seizure group and of the SE group are shown in Fig. 1A and B, respectively.

Rats in the isolated seizure group ($n = 7$) showed the first seizure on average $58.3 (\pm 17.6)$ min following the injection of 4AP whereas those in the SE group ($n = 6$) showed their first seizure on the EEG on average $25.3 (\pm 5.7)$ min after injection. The delay from the 4AP injection to the occurrence of the first seizure was, therefore, significantly shorter in rats that progressed into SE ($p < 0.05$). The average duration of the first electrographic seizure was $98 (\pm 19)$ s in the isolated seizure group and $89 (\pm 19)$ s in the SE group (Fig. 1C); these values were not significantly different. Finally, the mortality rate in the SE rats was 100%, while none of the animals in the isolated seizure group died after injection.

Next, we analyzed the occurrence of short epileptiform events (which resemble interictal spikes) occurring before the first seizure in order to establish if the difference in duration between 4AP injection and the first seizure between the two groups was mirrored by different interictal spike rates. As shown in Fig. 2A and B, interictal spikes were recorded from CA3 and EC in both groups. No significant differences in rates of occurrence were observed between groups, in any of the limbic areas recorded (Fig. 2C).

3.1. Rates of occurrence over time of high-frequency oscillations before, during, and after seizures

Only the first seizure of rats with good-quality recordings was selected for the analysis of HFOs. Consequently, the first seizure of 5 rats in the isolated seizure group and the first seizure in 5 rats of the SE group were analyzed for HFOs during the pre-ictal, ictal and post-ictal periods. In both experimental groups, the rate of occurrence of ripples and fast ripples during the preictal period was negligible and we could not identify any difference (data not shown). In contrast, different structure-specific distribution of HFOs could be identified during the ictal period in the isolated seizure and SE group. Specifically, fast ripple rates in the isolated seizure group were higher than ripple rates ($p < 0.05$) in CA3 and EC at the onset of the ictal discharge (Fig. 3A), whereas in the SE group this difference remained significant throughout the entire duration of seizures (Fig. 3B) ($p < 0.05$).

During the post-ictal period, in the isolated seizure group, fast ripples occurred at low rates in both CA3 and EC while during the last two thirds of the post-ictal period, ripples occurred at significantly higher rates compared to fast ripples, in CA3 (Fig. 4A, $p < 0.05$). In contrast, in the SE group, fast ripple occurrence in CA3 and EC was higher than that of ripples, and remained so throughout the entire duration of the post-ictal period ($p < 0.05$) (Fig. 4B). Since the rate of occurrence of both ripples and fast ripples was low during the post-ictal period, we also illustrate the relation between the absolute values of ripples and fast ripples during the post-ictal period in both the isolated seizure and the SE group (Fig. 4).

4. Discussion

The main findings of our study can be summarized as follows. First, the delay from the 4AP injection to the occurrence of the first seizure is significantly shorter in rats that progressed into SE than in those that generate an isolated seizure. Second, in the CA3 and EC of the SE group, fast ripple occurrence predominates over that of ripples throughout the entire duration of ictal events. Third, during the post-ictal period, in the SE group, fast ripples continue to predominate over ripples whereas in the isolated seizure group ripples tend to occur at higher rates compared to fast ripples. Therefore, patterns of HFOs during the first seizure after the systemic administration of 4AP can be used to predict its probability to progress into SE.

4.1. 4AP-induced ictogenesis in temporal lobe regions

We induced in this study two types of epileptiform activity with the systemic administration of 4AP: isolated seizures or SE. Moreover, in both groups, interictal spikes were observed in both temporal lobe regions (CA3 and EC). These results are therefore in line with previously

published studies showing that these temporal lobe regions are highly excitable following the administration of 4AP and can sustain ictogenesis (Lévesque et al., 2013; Salami et al., 2015). However, the onset pattern of the first seizure in all 4AP-injected animals could not be classified as either low-voltage fast onset or hypersynchronous, as we have previously reported (Lévesque et al., 2013; Salami et al., 2015). We were indeed expecting that the first seizure would show an LVF onset pattern. This discrepancy could be due to the fact that, in this study, we have analyzed only the first seizure after the injection of 4AP, whereas in previous studies (Lévesque et al., 2013; Salami et al., 2015) we have analyzed multiple seizures following injection. It is therefore possible that, early after the acute administration of a chemoconvulsant, seizure onset patterns are not clearly defined but gradually emerge over time depending on the neural network that is stimulated. Further studies are however needed in order to verify this hypothesis.

The delay between the injection of 4AP and the occurrence of the first isolated seizure found in this study was similar to what we have previously reported (Lévesque et al., 2013). However, we discovered that a shorter delay between 4AP administration and the first seizure occurred in animals progressing to SE thus suggesting that stronger network synchronization is present in these animals. Indeed, some animals may be more prone to develop seizures following chemoconvulsant administration; thus, as reported by Langer et al. (2011) in a different model epileptiform synchronization, some animals coming from the same supplier but from different breeding colonies may be more sensitive to 4AP. Different levels of stress due to the way they are handled at the institution may also have an impact on their seizure threshold (Lévesque et al., 2015).

4.2. High-frequency oscillations during ictal and post-ictal periods

We found that in the SE group, fast ripple rates were significantly higher compared to ripple rates, throughout the entire duration of seizures and during the post-ictal period. To our knowledge, this is the first study on the relation between HFO occurrence and SE. Although it is unknown if our findings can be applied to SE induced with other convulsive agents or to SE in humans, they indicate that patterns of HFOs differ between rats presenting with isolated seizures and those progressing to SE. The mechanisms underlying these two types of epileptiform events might thus differ. Indeed, since fast ripples are thought to reflect the abnormal synchronous firing of principal cells, independent of inhibitory neurotransmission (Bragin et al., 1999, 2011; Dzhala and Staley, 2004; Foffani et al., 2007; Ibarz et al., 2010), we are inclined to speculate that SE depends on the preponderant activation of pyramidal cell (glutamatergic) networks. In line with this hypothesis, it has been reported that the inhibition of hippocampal pyramidal neurons in the lithium–pilocarpine model delays the onset of SE (Sukhotinsky et al., 2013). Perforant-path stimulation also induces a SE that is dependent on the activity of NMDA receptors (Wasterlain et al., 2000).

In the isolated seizure group, rates of fast ripples progressively decreased over time during seizures and reached similar levels of occurrence compared to ripples during the post-ictal period, in both CA3 and EC. This is in contrast to the high occurrence of ripples previously observed in 4AP-induced seizures *in vivo* (Salami et al., 2015). However, as mentioned above, we analyzed in this study only the first seizure after the administration of 4AP and we

could not identify any pattern of onset that could indicate the preferential contribution of glutamatergic or GABAergic networks. It is thus possible that the first ictal discharge induced with 4AP *in vivo* depends on specific mechanisms of generation that differ from seizures occurring at later time points after injection. Further experiments are however needed to support this hypothesis.

In conclusion, our findings provide important insights into the role of HFOs as biomarkers of ictogenesis. This view may be extended to post-traumatic epileptic disorders (Agrawal et al., 2006), *i.e.*, fast ripples occurring during early ictal and post-ictal periods after an initial brain insult may identify abnormal and excessive conditions of neuronal network synchronization leading to SE. They may therefore constitute a target for early therapeutic interventions to stop SE and prevent epileptogenesis in patients.

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Fig. 1.

Representative seizures induced by 4AP in the isolated seizure and SE group. A: An example of the first recorded seizure in a rat in the isolated seizure group. The insets show the onset of the seizure on different expanded time scales. B: An example of seizures recorded in a rat in the SE group. The onset of the first seizure is shown on different expanded time scale. C: Bar graphs showing the time from the 4AP injection to the occurrence of the first seizure. In the SE group, the delay between the injection of 4AP and the occurrence of the seizure was significantly shorter compared to the isolated seizure group ($*p < 0.05$). D: Bar graph showing the duration of the first seizure in both groups. No significant differences were observed.

Fig. 2.

Interictal spikes during the pre-ictal period of both groups. A and B: Examples of interictal spikes recorded during the pre-ictal period in the isolated seizure (A) and SE group (B). C. Bar graph showing the rate of occurrence of interictal spikes in each region, for each group. No significant differences were observed between regions in any of the groups.

Fig. 3.

Temporal distribution of HFOs during the ictal period in the isolated seizure (A) and SE group (B). A: Temporal distribution of HFOs during the ictal periods of the first recorded seizure in the isolated seizure group. Note that the rate of occurrence of fast ripples is higher compared to the rate of occurrence of ripples at the beginning of the seizure in both CA3 and EC. B: Temporal distribution of HFOs during the ictal period of the first recorded seizure in the SE group. Note that the rate of occurrence of fast ripples is higher throughout the entire seizure in the SE group in both CA3 and EC. FR > R indicates that fast ripple rates are significantly higher compared to ripple rates ($p < 0.05$).

Fig. 4.

Temporal distribution of HFOs during the post-ictal period of the isolated seizure (A) and SE group (B). A: Temporal distribution of HFOs during the post-ictal period after the first recorded seizure in the isolated seizure group. Note that the rate of ripples is higher compared to that of fast ripples in CA3. B: Temporal distribution of HFOs during the post-ictal period after the first recorded seizure in the SE group. Note that the rate of occurrence of fast ripples is higher throughout the entire post-ictal period in both CA3 and EC (* $p < 0.05$). Insets show the relation between the absolute values of ripples and fast ripples during the post-ictal period in both the isolated seizure and the SE groups.