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Short communication

High frequency oscillations in epileptic rodents: Are we doing it right?

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HIGHLIGHTS

- We have analysed overlapping HFOs during spontaneous seizures in epileptic animals.
- Overlapping HFOs represent a small percentage of the total number of ictal HFOs.
- High rates occurred in the seizure onset zone, before low-voltage fast onset seizures.
- Overlapping HFOs occurred at high rates during hypersynchronous-onset seizures.
- These HFOs could represent novel oscillatory events providing additional information.

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ABSTRACT

Background: The detection of high-frequency oscillations (HFOs, ripples: 80–200 Hz, fast ripples: 250–500 Hz) is often based on considering ripples or fast ripples in isolation; overlapping ripples and fast ripples are excluded from further analysis. Here, we studied overlapping HFOs during spontaneous seizures in pilocarpine-treated animals.

New method: Spontaneous seizures (n = 6 animals) presented with either hypersynchronous- (HYP, n = 18) or low-voltage fast-onset (LVF, n = 21) pattern. Ripples and fast ripples overlapping by more than 30% were analysed.

Results: Overlapping HFOs could show a unimodal power spectrum between 80–500 Hz (n = 188, 58.9%) or a bimodal power spectrum, with peaks in power between 80 and 200 Hz and between 250 and 500 Hz (n = 131, 41.1%,). Overlapping HFOs occurred at higher rates during HYP seizures compared to the pre-ictal period in seizure onset zones ($p < 0.001$) and regions of secondary spread ($p < 0.001$). When comparing HYP and LVF seizures, we found that overlapping HFOs occurred at higher rates before LVF seizures ($p < 0.05$) compared to HYP seizures but, during the ictal period, HYP seizures showed higher rates of overlapping HFOs than LVF seizures ($p < 0.001$).

Comparison with existing methods: We have analysed overlapping ripples and fast ripples shortly before and during seizures.

Conclusions: Although overlapping ripples and fast ripples represent a minority of HFOs, they may provide additional information on the excitability of neuronal networks that generate seizures in animal models and patients presenting with mesial temporal lobe epilepsy.

1. Introduction

High-frequency oscillations (HFOs, ripples: 80–200 Hz, fast ripples: 250–500 Hz) occur in the EEG of patients mesial temporal lobe epilepsy (MTLE) and in animal models mimicking this condition (Jefferys et al., 2012). HFOs have been recorded in association with interictal spikes and seizures and are believed to reflect the abnormal neuronal activity generated by the epileptic tissue (Jefferys et al., 2012). We have indeed found in the pilocarpine model of MTLE that seizures with specific onset patterns are predominated by the occurrence of either ripples or fast ripples (Lévesque et al., 2012). Hypersynchronous-onset (HYP) seizures are characterized with an onset pattern of focal spiking at a frequency of ~2 Hz and are mainly associated with fast ripples. In contrast, low-voltage-fast onset (LVF) seizures, which are characterized by an initial, isolated spike followed by low-amplitude fast activity, present with a predominance of ripples. Such predominance of either ripples

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or fast ripples during LVF and HYP seizures, respectively, suggests that these HFOs are generated by the activity of distinct temporal lobe networks. This hypothesis was supported by a recent *in vitro* study in which optogenetic stimulation of interneurons triggered LVF ictal-like discharges (with increased occurrence of ripples) whereas stimulation of principal cells triggered HYP ictal-like discharges (with predominant fast ripples) during application of 4-aminopyridine (Shiri et al., 2016).

HFOs are often considered as genuine events when they are only visible in the ripple (80–200 Hz) or fast ripple frequency band (250–500 Hz). Therefore, HFOs occurring simultaneously in both ripple and fast ripple frequency band were not taken into consideration in many previous studies (Behr et al., 2017; Lévesque et al., 2012; Perucca et al., 2013; Salami et al., 2015). However, a recent study performed in epileptic patients undergoing brain surgery, has shown that surgical removal of brain areas in which ripples co-occurred with fast ripples could lead to a 100% seizure freedom (Fedele et al., 2017). In this study, we have therefore investigated overlapping ripples and fast ripples during seizures occurring in pilocarpine-treated epileptic rats. Specifically, we aimed at establishing: (i) whether overlapping ripples and fast ripples occur at significantly higher rates during the pre-ictal or ictal period of seizures, in seizure onset zones and in regions of secondary spread; (ii) whether they are associated with specific seizure onset patterns and (iii) whether overlapping HFOs reflect a single event type that includes multiple frequencies or rather reflect the co-occurrence of a ripple and a fast ripple.

2. Materials and methods

2.1. Animal preparation

We used the same methods described by Lévesque et al. (2012). Male Sprague-Dawley rats (250–300 g; Charles-River (St-Constant, QC, Canada)) were let habituate for 72 h before pilocarpine treatment. Animals were housed at 22 (±2) °C under 12 h light/12 h dark cycle with food and water *ad libitum*. All procedures were approved by the Institutional Animal Care Committee of McGill University. Every effort was made to minimize the number of animals used and their suffering.

2.2. Pilocarpine treatment

Scopolamine methylnitrate (1 mg/kg i.p.; Sigma-Aldrich, Canada) was administered 30 min before pilocarpine hydrochloride (380 mg/kg, i.p.; Sigma-Aldrich, Canada) to induce a *status epilepticus* (SE) that was stopped after 1 h using diazepam (5 mg/kg, s.c.; CDMV, Canada) and ketamine (50 mg/kg, s.c.; CDMV, Canada). The mortality rate after pilocarpine injection was approximately 15%.

2.3. Implantation of depth electrodes

Three days after SE, rats underwent surgery for the implantation of bipolar depth electrodes. Rats were anesthetized with isoflurane (3%) in 100% O2. Four bipolar electrodes (20–30 kΩ; 4–10 mm length; distance between exposed tips: 500 μm; MS303/2-B/spc, Plastics One, VA, USA) were implanted in the CA3 subfield of the ventral hippocampus (AP: −4.4, ML: −4, DV: −7.8), the medial entorhinal cortex (AP: −6.6, ML: −5.2, DV: −6.8); the ventral subiculum (AP: −6.8, ML: +4, DV: −6), and the dentate gyrus (AP: −4.4, ML: +2.4, DV: −3.4) (Paxinos and Watson, 1998). Four stainless steel anchor screws (2.4 mm length) were fixed to the skull and a fifth electrode was used as reference (5–10 kΩ), after removal of insulating material, and placed under the frontal bone. Electrodes, the reference and anchor screws were embedded in dental cement, which made a head-plug that was later connected to a tethered recording system. Movements of the animal were not restrained by the tethered system. Ketoprofen (5 mg/kg, s.c. Merail, Quebec, Canada), buprenorphine (0.01–0.05 mg/kg, s.c. repeated every 12 h; CDMV, Quebec, Canada) and 2 ml of 0.9% sterile saline were given up to 3 days after surgery. Continuous EEG-video recordings were performed 24 h/day. EEG signals were amplified *via* an interface kit (Mobile 36ch LTM ProAmp, Stellate, Montreal, QC, Canada), low-pass filtered at 500 Hz and sampled at 2 000 Hz. Throughout the recordings, animals were placed under controlled conditions (22 ± 2C, 12-h light/dark schedule) and provided with food and water *ad libitum*.

2.4. Classification of seizure onset patterns

Seizures were identified with the ICTA-D seizure detector (Harmonie; Stellate). Seizure onset was identified by the appearance of fast activity at 15–20 Hz (Fig. 1 A, arrowheads) (Lévesque et al., 2012). Seizures were classified in two groups according to their onset pattern (Behr et al., 2017; Lévesque et al., 2012; Velasco et al., 2000). LVF seizures were characterized by the occurrence of a positive- or negative-going spike that was followed by the occurrence of low-amplitude, high-frequency activity whereas HYP seizures were characterized at onset by a pattern of focal spiking at a frequency of ∼2 Hz. Seizures that could not be classified as HYP or LVF were termed “unclassified”.

2.5. Seizure onset zones

We analyzed seizure onset zones involved in HYP and LVF seizures following the classification used by Lévesque et al. (2012). We termed: “CA3” those seizures initiating in the CA3 subfield only; “CA3+” those seizures characterized by a simultaneous onset in CA3 and another region; “widespread” seizures those initiating simultaneously in all regions; and “CA3–” those seizures that did not involve CA3. Since categorical variables were used, we employed chi-square tests for statistical comparisons.

2.6. Analysis of high-frequency oscillations

When a seizure was detected, the EEG signal was extracted and exported to Matlab (The Mathworks, Natick, MA) for off-line analysis. HFOs were detected using the method previously published by Lévesque et al. (2012). Briefly, raw EEG recordings were band-pass filtered in the 80–200 Hz and in the 250–500 Hz frequency range. We used a 10 s period from 50 s to 40 s before seizure onset as reference period for signal normalization. Each channel was then normalized to its own reference period. To be considered as an HFO candidate, oscillatory events in the ripple and fast ripple frequency range had to show at least four consecutive cycles having amplitude of 3 SD above the mean of the reference period.

Overlapping events were defined as the co-occurrence of a ripple and a fast ripple that overlapped by at least 30%. The percentage of overlap was calculated by dividing the time period during which a ripple and a fast ripple overlapped by the time period of the entire overlapping HFO. HFOs were analysed during a 30 s pre-ictal period and during the ictal period. To account for differences in seizure duration, each seizure was transformed into a scale from 0 (start of the seizure) to 100 (end of the seizure). Rates of overlapping HFOs were calculated for the pre-ictal and ictal period. Average rates of overlapping HFOs were then obtained for regions considered as seizure onset zones and for regions of secondary spread. Therefore, we did not compare rates of overlapping HFOs between regions (CA3, entorhinal cortex, subicuum and dentate gyrus) but instead analysed their distribution in seizures onset zones and regions of secondary spread, as it was performed in Lévesque et al. (2012) for