

## Spike ripples in striatum correlate with seizure risk in two mouse models



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### ABSTRACT

Epilepsy biomarkers from electroencephalogram recordings are routinely used to assess seizure risk and localization. Two widely adopted biomarkers include: (i) interictal spikes, and (ii) high frequency ripple oscillations. The combination of these two biomarkers, ripples co-occurring with spikes (spike ripples), has been proposed as an improved biomarker for the epileptogenic zone and epileptogenicity in humans and rodent models. Whether spike ripples translate to predict seizure risk in rodent seizure models is unknown. Further, recent evidence suggests ictal networks can include deep gray nuclei in humans. Whether pathologic spike ripples and seizures are also observed in the basal ganglia in rodent models has not been explored. We addressed these questions using local field potential recordings from mice with and without striatal seizures after carbachol or 6-hydroxydopamine infusions into the striatum. We found increased spike ripples in the interictal and ictal periods in mice with seizures compared to pre-infusion and post-infusion seizure-free recordings. These data provide evidence of electrographic seizures involving the striatum in mice and support the candidacy of spike ripples as a translational biomarker for seizure risk in mouse models.

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### 1. Introduction

Epilepsy biomarkers from electroencephalogram (EEG) recordings are routinely used to assess seizure risk and localize the seizure onset zone to optimize neurosurgical interventions [1]. Two widely adopted biomarkers include: (i) interictal spikes, and (ii) high frequency oscillations (HFOs) [1,2]. Spikes are large voltage fluctuations specific to the diagnosis of epilepsy or an increased risk of spontaneous seizures [3], but are spatially diffuse and only partially concordant with the epileptogenic zone [4]. Ripples are brief bursts of 80–250 Hz [5] low-amplitude HFOs that are closely linked to the epileptogenic zone [1–4] and may reflect the severity of epilepsy [6]. However, ripples are also common in physiologic

processes [7] and therefore not specific for epilepsy. The combination of these two biomarkers, spike ripples (e.g., ripples co-occurring with spikes), has been proposed as an improved biomarker for the epileptogenic zone [6–8] and epileptogenicity [9].

The neuronal and subcellular mechanisms that support spikes, ripples, and the interaction between them are not understood but could be further elucidated in animal models. In rodent models of seizures and epilepsy, interictal spikes [10,11], physiologic and pathologic ripples [12,13], and the co-occurrence of these events have been reported [14–16]. Whether the combined epileptogenic biomarker, spike ripples, predicts increased seizure risk in rodent seizure models is unknown. Further, although recent reports in humans and rodents have identified ictal activity in deep gray nuclei, including the thalamus and the basal ganglia [17–19], seizure models in mice have primarily focused on hippocampal and cortical ictal events. Whether the basal ganglia produce pathologic spike ripples and seizures remains poorly understood.

To address these questions, we evaluated recordings from mice observed to have striatal seizures after carbachol [20] or

Abbreviations: EEG, electroencephalogram; HFO, high frequency oscillation; 6-OHDA, 6-hydroxydopamine; LFP, local field potential.

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6-hydroxydopamine (6-OHDA) [21] infusions. Using a validated spike ripple detector applied to local field potential (LFP) data, we found increased spike ripples in the interictal and ictal periods in mice with symptomatic electrographic seizures compared to pre-infusion and post-infusion recordings in mice without seizures. This small dataset provides evidence of electrographic seizures involving the striatum in mice and suggest that spike ripples may provide a promising and sensitive translational biomarker for seizure risk in mouse models.

## 2. Materials and methods

### 2.1. Mouse models

All animal procedures were approved by the Boston University Institutional Animal Care and Use Committee. Seventeen adult mice aged 8–12 weeks, both male and female, Chat-Cre:GM24Gsat or PV-CreB6;129P2-Pvalbtm1(cre)Arbr/J, on the genetic background of C57BL/6, were surgically implanted with an electrode (120um diameter stainless steel wire with polyimide) attached to a drug infusion and guide cannula in the dorsal striatum (anteroposterior + 0.5 mm, mediolateral 1.8 mm, dorsoventral –1.6 mm from brain surface). All LFP recordings were made with animals awake and head fixed on a spherical treadmill to permit locomotion [22]. Pre-infusion recording sessions were performed in all mice prior to infusion. Six mice received intracranial carbachol infusion; three of the mice that did not develop seizures also received 6-OHDA infusion 14–21 days after carbachol infusion. Eleven additional mice received intracranial 6-OHDA infusion alone. All infusions were made in the striatum about 300–500um away from the LFP recording electrode. No mice were observed to have seizures clinically or electrographically before either chemical infusion. Seizures were identified when epileptiform activity lasting for 10 seconds or longer with progression in either amplitude or frequency was observed in electrographic recordings.

For the mice that received carbachol or 6-OHDA infusions, we recorded up to five or three 10-minute sessions of pre-infusion LFP recordings, separated by 3 or 7 days, respectively. After the last pre-infusion session, we infused carbachol (1  $\mu$ L, 0.2ug/uL) or 6-OHDA (1  $\mu$ l of 5  $\mu$ g/ul) and then recorded LFP for ~20 minutes or intermittently for 10–20 minutes over the next 2–4 days, respectively. Of the 17 mice tested in these experimental conditions (three tested in each condition), two developed confirmed electroclinical seizures post-infusion (carbachol: M4003; 6-OHDA: M8815). These post-infusion recordings were used to quantify spike ripple events during the ictal and interictal periods (Fig. 1A). Ictal periods are defined from the onset to termination of the electrographic seizure. Interictal periods are defined as all recording sessions in mice with seizures post-chemical infusion, excluding the ictal periods.

Because no seizures were detected or expected prior to chemical infusions, all recordings from all mice before carbachol ( $n = 14$ , 7 male) or 6-OHDA infusion ( $n = 6$ , 4 male, 2 unknown) were included as pre-infusion recordings ( $n = 20$ ). For post-carbachol or 6-OHDA infusion data, mice with definite electrographic seizures captured during at least one of their LFP recordings were included for the analysis of ictal ( $n = 2$ ) and interictal ( $n = 2$ ) data; mice without electrographic seizures were included as post-infusion seizure-free ( $n = 18$ ) data.

### 2.2. Data processing

LFP data were sampled at 3052 Hz and referenced to a ground electrode on the skull surface of the contralateral hemisphere. Epochs with large movement artifacts lasting longer than 10 s were

manually identified and removed. All data were filtered with a 0.5–300 Hz bandpass filter and 60 Hz notch filter. Extreme amplitude deviations were automatically detected (minimum peak distance is 20 ms; minimum peak prominence is 2 times the mean of the absolute value of the data). The detected peaks that exceeded 5 standard deviations of the mean of all detected extrema within a recording session were removed ( $\pm 100$  ms).

### 2.3. Spike ripple detector

To increase efficiency and reliability of detection, data were analyzed using an automated spike ripple detector [9,23] (<https://github.com/Mark-Kramer/Spike-Ripple-Detector-Method>). The detector has been demonstrated to have high accuracy for spike ripple events in human scalp EEG data with high intra- and inter-rater reliability [9,23].

### 2.4. Statistical analysis

We hypothesized that the spike ripple rate would provide a biomarker for seizure risk in mouse LFP and therefore be highest in the ictal period, and higher in the ictal and interictal periods compared to pre-infusion periods and post-infusion periods in seizure-free mice. This hypothesis was tested by modeling spike ripple rate (generalized linear model with Poisson distribution, log link, and estimated dispersion) with categorical predictor of group (interictal, ictal, and combined pre-infusion and post-infusion periods in seizure-free mice).

## 3. Results

### 3.1. Evidence of striatal seizures in two mouse models

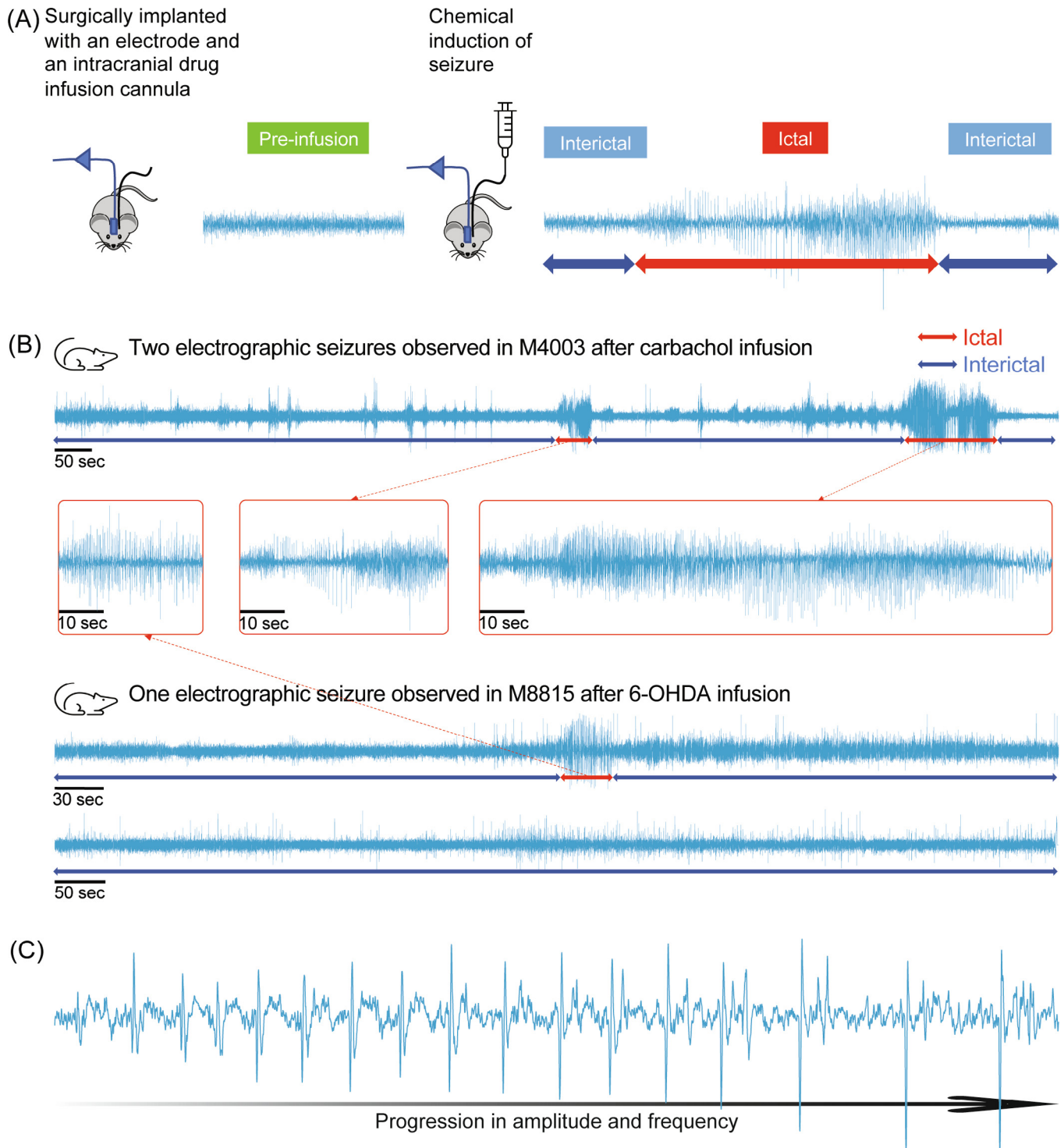
During the post-infusion period, some mice were observed to have clinical seizures, characterized by behavioral arrest and tonic and jerking of the limbs contralateral to the infusion. Upon review of the LFP data, we identified three organized electrographic seizures from mouse striatum following infusion of carbachol (1 mouse, 2 seizures captured) or 6-OHDA (1 mouse, 1 seizure captured) (Fig. 1B). An example seizure showing progression in amplitude and frequency is shown in Fig. 1C. These examples provide evidence that intracranial striatal infusion of carbachol or 6-OHDA can induce electrographic seizures in the mouse striatum.

### 3.2. Spike ripples following striatal infusion of 6-OHDA or carbachol

For the carbachol group ( $n = 6$ ), we examined a total of 107.3 minutes of pre-infusion data before carbachol infusion from all 6 mice, and 19.23 (2.87) minutes of interictal (ictal) data from mouse 4003. For the 6-OHDA group ( $n = 14$ ), we analyzed a total of 380.0 minutes of pre-infusion data from all 14 mice before 6-OHDA infusion, and 26.95 (0.58) minutes of interictal (ictal) data in mouse M8815. We found spike ripples with temporal and spectral features matching those observed in humans [9,23] during the interictal periods (example in Fig. 2A) and ictal periods (example in Fig. 2B) in both mice with seizures.

### 3.3. Increased spike ripple rate correlates with seizure risk

The spike ripple rates for all mice are shown in Fig. 2C. We detected no difference in spike ripple rate in seizure-free mice pre-infusion compared to post-infusion ( $p = 0.13$ ). Spike ripple rate was higher in the ictal comparing to interictal period ( $p = 0.04$ ) or

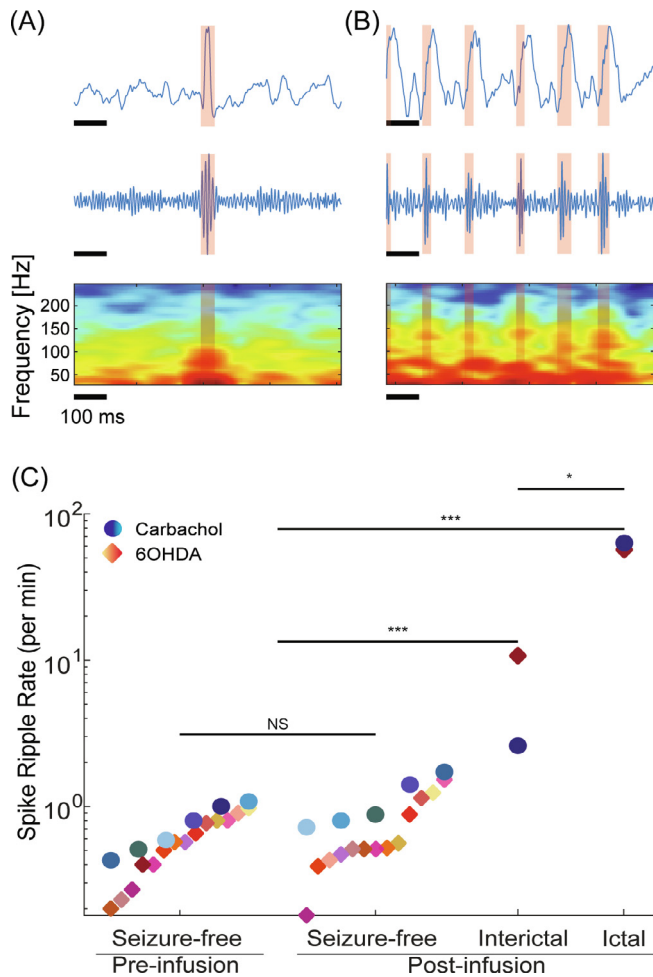


**Fig. 1.** (A) Each mouse was surgically implanted with a recording electrode and an intracranial drug infusion cannula. LFPs were recorded in up to three pre-infusion sessions. Animals then received either carbachol or 6-OHDA infusion through the cannula into the dorsal striatum, and subsequently recorded during ictal (red) and interictal (blue) periods. (B) LFP recordings from the two mice with post-infusion electrographic seizures (upper, after carbachol infusion; lower, after 6-OHDA infusion). Ictal (red) and interictal (blue) periods are indicated. Ictal events are expanded in the insets. (C) An example seizure from M4003. Epileptiform activity with progression in both amplitude and in frequency. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

seizure-free periods ( $p = 4e - 32$ ). Spike ripple rate was increased in the interictal ( $p = 1e - 14$ ) period compared to seizure-free periods. When the 6-OHDA and carbachol striatal mouse models were analyzed separately, spike ripple rates remained higher during the interictal and ictal periods compared to pre-infusion and post-infusion periods in seizure-free mice, in both models ( $p < 4e - 3$ ).

#### 4. Discussion

We identified spike ripples in mice with and without symptomatic seizures and found that the spike ripple rate was increased in the setting of increased seizure risk. These results highlight spike ripples as a promising translational biomarker for seizure



**Fig. 2.** (A) Example spike ripple detection from mouse striatal LFP data during an example interictal period. For each detected spike ripple example, the display elements include: the raw LFP (top), the bandpass filtered LFP (~100–300 Hz, middle), and the spectrogram (bottom) with power in decibels (color). In each figure, the red highlighted area indicate the time interval of a detected spike ripple event. (B) Example spike ripple detection during an ictal period. In this example, multiple spike ripples can be observed. (C) The spike ripple rate during pre-infusion recordings and post-infusion periods in mice with carbachol (filled circle) or 6-OHDA (filled diamond) infusion. Each pair of pre-infusion and post-infusion markers represents 1 mouse. Spike ripple rate measured from the same mice are shown in the same color. Comparison results between groups are indicated (NS:  $p > 0.05$ , \*:  $p < 0.05$ , \*\*\*:  $p < 0.001$ ).

risk and localization of the seizure network in rodent models. The mechanisms generating spike ripples are unknown and challenging to study in humans [24,25]. *In vitro* studies have found conflicting results, reporting that spikes and ripples may suppress [24] or promote [25] each other's occurrence. Future work evaluating this biomarker in a larger population of rodent models of epilepsy are required to identify the neuronal mechanisms responsible for generating these pathologic events utilizing novel invasive imaging tools, in order to identify improved targets for treatment [26].

This study involved secondary use of data from mice recorded for a separate project [27]. As seizures were observed in a subset of animals, we took the opportunity to evaluate spike ripples as a biomarker for seizure risk in this available cohort rather than require new animals. Notably, interictal activity and provoked seizures were recorded in the striatum in both the carbachol and 6-OHDA mouse models tested, though with low-yield at the doses used. Prior work has demonstrated that carbachol and 6-OHDA can both reduce seizure thresholds in rodent models [20,21]

including in the basal ganglia [28] presumably due to a relative choline excess and dopamine depletion, and have reported higher yields with higher doses. Our observations highlight the potential for interictal and ictal involvement of the neuronal populations in the basal ganglia, the need for further studies to evaluate the incidence of spontaneous seizures in the striatum and mechanisms of striatal seizures in both humans [17,18] and rodent models, and the question of whether these striatal seizures originate in the striatum or are propagated from other brain regions such as the cortex. Further, neuromodulation targeting brain structures is becoming an increasingly popular treatment for drug-resistant epilepsy [29,30]. This includes electrode placement in the centromedian nucleus which is intimately connected to the basal ganglia [31,32]. Therefore, identification of relevant interictal biomarkers in these regions are increasing important. Our results suggest that spike ripples may provide a promising interictal biomarker for seizure risk in the deep gray nuclei. Confirmation of these findings should be investigated in larger samples and other animal models.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Declarations of interest

None.

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