Title: The Entrainment of the Substantia Nigra During Temporal Lobe Seizures is Dependent on the Seizure Onset Pattern

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Abstract:

Rationale: Temporal lobe (TL) epilepsy is the most common form of drug-resistant epilepsy. While the limbic circuit and the structures composing the TL have been a major focus of human and animal studies on TL seizures, there is also evidence suggesting that the basal ganglia have an active role in the propagation and control of TL seizures. Studies in patients have shown that TL seizures can cause changes in the oscillatory activity of the basal ganglia when the seizures spread to extratemporal structures. Preclinical studies have found that inhibition of the substantia nigra pars reticulata (SN), a major output structure of the basal ganglia, can reduce the duration and severity of TL seizures in animal models. These findings suggest the SN plays a role critical in the maintenance or propagation of TL seizures. Two stereotyped onset patterns commonly observed in TL seizures are low-amplitude fast (LAF) and high-amplitude slow (HAS). Both onset patterns can arise from the same ictogenic circuit, however seizures with LAF onset pattern typically spread farther and have a larger onset zone than HAS. Therefore, we would expect LAF seizures to entrain the SN more so than HAS seizures. Here, we use a nonhuman primate (NHP) model of TL seizures to confirm the implication of the SN in TL seizure and to characterize the relationship between TL seizure onset pattern and the entrainment of the SN.

Methods: Recording electrodes were implanted in the hippocampus (HPC) and SN in 2 NHPs. One subject was also implanted with extradural screws for recording activity in the somatosensory cortex (SI). Neural activity from both structures was recorded at a 2 kHz sampling rate. Seizures were induced by intrahippocampal injection of penicillin, which produced multiple spontaneous, nonconvulsive seizures over 3-5 hours. The seizure onset patterns were manually classified as LAF, HAS or other/undetermined. Across all seizures, spectral power and coherence were calculated for the frequency bands 1–7 Hz, 8–12 Hz and

13–25 Hz from/between both structures and compared between the 3 seconds before the seizure, the first 3 seconds of the seizure, and the 3 seconds before seizure offset. These changes were then compared between the LAF and HAS onset patterns.

Results: During temporal lobe seizures, the 8–12 Hz and 13–25 Hz power in the SN along with the 1–7 Hz and 13–15 Hz power in the SI was significantly higher during onset than before the seizure. Both the SN and SI had an increase in coherence with the HPC in the 13–25 Hz and 1–7 Hz frequency ranges, respectively. Comparing these differences between LAF and HAS, both were associated with the increase in the HPC/SI coherence, while the increase in HPC/SN increase was specific to LAF.

Conclusion: Our findings suggest that the SN may be entrained by temporal lobe seizures secondary to the SI during the farther spreading of LAF seizures, which supports the theory that the SN plays a role in the generalization and/or maintenance of temporal lobe seizures and helps explains the anti-ictogenic effect of SN inhibition.

1. Introduction

Temporal lobe epilepsy is the most common form of drug-resistant partial epilepsy [1]. A major focus of human and animal studies on temporal lobe epilepsy has been the limbic circuit and the structures composing the temporal lobe. However, there is also evidence suggesting an active role of the basal ganglia (BG) in the propagation and control of temporal lobe seizures. The BG network is a complex grouping of interconnected structures that receive diverse and topographically organized inputs from the cortex and thalamus [2, 3] and is indirectly connected to the hippocampus (HPC), and amygdala [4-7]. Studies using intracranial recording have identified changes in oscillatory activity of basal ganglia structures including the putamen, pallidum, and caudate when temporal lobe seizures propagated beyond the temporal lobe [8, 9] and in the putamen during temporal lobe seizures with ictal limb dystonia [10]. In addition, a PET study in patients with temporal and extratemporal epilepsy found a decreased uptake of [^{18}F] fluoro-L-dopa in the SN, caudate, and putamen compared to healthy controls [11].

As one of the major outputs of the BG, the substantia nigra (SN) has been proposed to be a critical node responsible for the maintenance of temporal lobe seizures. In rodents, direct and indirect inhibition of the SN results in control of amygdala-kindled seizures [12-14]. Moreover, inhibition of the SN, particularly the anterior SN has been shown to anti-ictogenic across a range of different models of focal and generalized seizures [15-21]. Similarly, low frequency stimulation of the SN has been found to suppress seizures in a cat penicillin (PCN) hippocampal seizure model [22].

The variability in whether a temporal lobe seizure propagates through the SN or other basal ganglia structures may be mediated by the seizure onset pattern. Two categories of seizure are commonly reported in patients with temporal lobe epilepsy based on the appearance and morphology of the onset pattern. The low amplitude fast (LAF) onset pattern is characterized by low amplitude oscillations in the beta to gamma range that slowly increase as

the seizure progresses, whereas the high amplitude slow (HAS) onset pattern consists of high amplitude slow oscillations usually below the alpha range [23, 24]. While both seizure onset patterns can arise from the same ictogenic circuit, seizures with the LAF onset pattern typically spread farther and have a larger seizure onset zone [23-27]. Therefore, we would expect that the seizures with the LAF onset pattern would be more likely to entrain the SN compared to the seizures with the HAS onset pattern.

So far, the relationship between the onset pattern of temporal lobe seizures and the involvement of the BG has not been established. In this study, we characterize the involvement of the SN during temporal lobe seizures induced in two nonhuman primates (NHP) and test the hypothesis that the entrainment of the SN is dependent on the seizure onset pattern.

2. Materials and Methods

2.1 Overview

We recorded local field potential (LFP) activity from the HPC and SN in two NHPs during temporal lobe seizures induced by local injection of penicillin in the HPC. Using these recordings, we characterized the changes in the oscillatory activity of the SN during the ictal period and how the coherence between the HPC and SN changes depending on the seizure onset pattern.

2.2 Animals, surgical procedures

Two rhesus macaques (macaca mulatta; 6-10 kg; one male, one female) were used for electrophysiological recording of the HPC and SN during temporal lobe seizures. The animals were pair-housed with other animals and had free access to food and water. All experiments were performed in accordance with the United States Public Health Service Policy on the humane care and use of laboratory animals, including the provisions of the “Guide for the Care

and Use of Laboratory Animals" [28]. All studies were approved by the Institutional Biosafety and Animal Care and Use Committees of Emory University. The animals were first habituated to be handled by an experimenter and to sit in a primate chair. They then underwent aseptic surgery under isoflurane anesthesia (1-3%). Both animals had a recording chamber (Crist Instruments, Hagerstown, MD; inner chamber diameter 18 mm) implanted on their right side. The chamber was placed vertically in the coronal plane to permit simultaneous access to the HPC and the SN for recording and injection (Figure 1B). NHP 2 had an additional electrode lead chronically implanted in the HPC (D08-15AM, 2 mm contact length, 1.5 mm between contacts, DIXI medical, France) and 2 ECoG electrodes located in the ipsilateral somatosensory cortex (SI).

2.3 Seizure induction

Seizures were induced by an intrahippocampal injection of PCN diluted in sterile water (Penicillin G sodium salt, and sterile water for injection from Sigma-Aldrich, St. Louis, MI). Final concentration of PCN was 1,000 I.U./μl and for each session a dose of 6,000-10,000 I.U. of PCN was delivered at a rate of 1 μl/min using an injection system connected to a mechanical pump and microsyringe (CMA, Harvard Biosciences Inc, Krista, Sweden). At the end of the injection, the system was clamped to prevent any additional PCN leakage and carefully raised. Details of the model have been previously described [29]. Each PCN injection induced multiple spontaneous self-terminating seizures over a period of 4-6 hours and injections were separated by at least 2 weeks. No spontaneous behavioral seizures were observed in between injections. The seizures induced were focal and not associated with obvious clinical motor symptoms. No oral automatisms were noted during ictal events.

2.4 Electrophysiological mapping and recording

Recordings were made while the animals were seated in a primate chair with their head fixed but body and limbs were free to move. Neural activity was recorded using a Cerebus data acquisition system (Blackrock Neurotech, Salt Lake City, UT). A post-surgery MRI was performed to orient the chamber and the locations of SN and HPC were confirmed by extracellular activity recorded with tungsten microelectrodes (FHC, Bowdoinham, ME; Z=0.5-1.0MΩ at 1kHz). The dura was pierced with a guide cannula and the electrode lowered into the brain with a microdrive (Nan instrument, Nof Hagalil, Israel). After delineating the SN and HPC borders, both structures were targeted for simultaneous LFP recordings with a 12-contact electrode consisting of 4 rings divided in 3 segments (outer diameter 600μm; inter-contact separation, 500μm; impedance, 25-35 kΩ; Heraeus Medical Components, St Paul, MN). On each recording day, one of electrode was placed in the SN and the other in the HPC for simultaneous recordings from the two structures. The injection system then was lowered into the HPC, and seizures were induced. For NHP 2, ECoG from the SI was simultaneously recorded using a bipolar montage. LFP and ECoG signals were sampled at 2 kHz synchronized with the video of the animal.

2.5 Data processing and analysis

2.5.1 Pre-processing

Data were imported in Spike 2 interface (CED, Cambridge, UK) for offline annotation of ictal activity. Ictal activities were manually marked based on electrophysiological signal. We recorded 14 and 59 seizures respectively in NHP 1 and NHP 2. Subsequent analysis was performed using MATLAB (MATLAB R2021a, The Mathworks, Natick, MA, USA). The signal from each electrode contact was first normalized, then the 3 directional contacts positioned at the same level were averaged to mimic a configuration analogous to a clinical concentric 4x1 electrode.

Finally, the signal from the contact located in the ventral part of the structure was subtracted from the signal in the dorsal part to obtain a bipolar recording signal in each structure.

2.5.2 Seizure classification

The seizure onset pattern was classified by a trained experimenter based on the first 3 seconds of the seizure recorded in the HPC. We identified 2 common seizure onset patterns. The low amplitude fast (LAF) activity onset pattern is characterized by low amplitude oscillations in the beta range that increases in amplitude as the seizure progresses. The high amplitude slow (HAS) onset pattern is characterized by high amplitude low frequency oscillations at seizure onset [23, 30]. The distribution of seizure onset patterns was compared between animals were using a Chi-square test.

2.5.3 Analysis

Simultaneous recordings from the HPC, SN, and SI were analyzed using the Chronux analysis software [31]. The individual power spectrogram and cohereogram between signals was calculated using the multi-tapper time-frequency method (moving window of 1 second and a window shift of 0.25 seconds). We extracted the power spectral density from the 3 seconds of signal preceding the seizure (pre-ictal), the 3 first seconds of the seizure onset and in the 3 last seconds of the seizure offset. The change in power and coherence were analyzed in 3 frequency bands: 1–7 Hz, 8–12 Hz and 13–25 Hz. These frequency bands were chosen based on the spectral activity of our PCN-induced seizure and previous clinical studies showing a cut off between LAF and HAS at 8 Hz [32]. All quantitative data were expressed as mean ± SEM. The statistical reliability of the differences between periods of interest (preictal, seizure onset, and seizure offset) was assessed using a Friedman repeated measures test for paired quantitative data and Dunnett's test for post hoc analysis. Comparisons between LAF and HAS

seizures were performed with a Mann-Whitney Rank Sum test. Statistical values were corrected for multiple comparisons using the Bonferroni method.

2.6 Histological verification of electrode placement

After completion of the experiment, the animals were euthanized with an overdose of pentobarbital sodium (100 mg/kg, i.v.) and transcardially perfused with cold oxygenated Ringer's solution, followed by a fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in a phosphate buffer (PB) solution. After perfusion, the brains were removed from the skull, cut coronally into 30 mm thick blocks, and post-fixed overnight in 4% paraformaldehyde. The blocks were then cryoprotected in ascending sucrose solution (10, 20 and 30% in PBS), cut into 50 µm-thick coronal sections using a freezing microtome and collected in series. Sections encompassing the SN from one series were immunostained using a tyrosine hydroxylase antibody, a marker of dopaminergic neurons to identify the position of the SN. Sections were rinsed in phosphate buffered saline (PBS; P3813, Sigma Aldrich, St. Louis, MO), permeabilized in PBS containing 0.3% hydrogen peroxide (H1009, Sigma Aldrich) and 0.1% of Triton-X (T8787, Sigma Aldrich) for 20 minutes with no shaking. Following several PBS rinses, sections were then incubated in 4% normal goat serum/PBS (NGS; 017-000121; Jackson Immunoresearch Laboratories) for 30 minutes at RT with shaking, rinsed in PBS, and incubated overnight in rabbit anti-TH antibodies (cat#P40101-150, Pel-Freez, Rogers, AR) at 4C with shaking. The next day, sections were rinsed and incubated in biotin SP conjugated donkey anti-rabbit IgG antibodies (cat#711-065-152, Jackson Immunoresearch Laboratories) in PBS containing 2% NGS at RT for 1hr with shaking, followed by additional rinses and an overnight incubation in avidin biotin complex (Vectastain Elite ABC kit HRP, standard, cat#PK-6100, Vector Laboratories). The next day sections were rinsed in PBS and incubated in a solution containing 3’3’-diaminobezidine tetrahydrochloride hydrate (DAB; cat#D3737, Tokyo Chemical

Industry) and hydrogen peroxide for 12 minutes at RT with shaking. Stained sections were mounted on gelatin coated glass slides, dried and coverslipped with Permount (SP15-100, Fischer Chemical). Sections through the SN from an adjacent series were mounted on glass slides and stained with cresyl violet to verify the electrode tract.

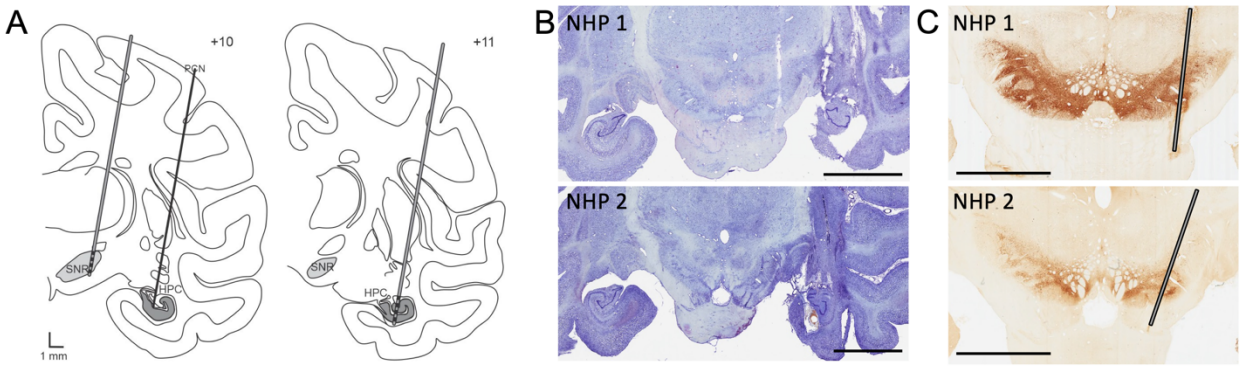

Figure 1: Electrode targeting. Schematic representation of the electrode placement in the SN and HPC as well as the PCN injection site in the HPC. B. Nissl stained sections showing the PCN injection site and electrode insertion tracts in the HPC for NHP 1 and 2. C) TH staining showing the electrode tract in the SN for NHP 1 and 2. Abbreviations: Substantia Nigra Reticulata (SNR), Hippocampus (HPC), Penicillin (PCN). Scale bars: B: 9 mm, C: 6 mm.

3. Results

3.1 Early involvement of the SN during TL seizure

In both animals, HPC activity was characterized by an increase in the 1–7 Hz, 8–12 Hz and 13–25 Hz frequency bands at seizure onset (Figure 2 and Supplementary Table 1). These oscillatory activities decreased at the end of the seizures but were still significantly higher in the 13–25 Hz range for both animals and in the 8–12 Hz for NHP 1. In contrast, while the power of the HPC in the low frequency band 1–7 Hz was increased at the beginning of the seizure, it returned to pre-ictal levels by the end of the seizure. No change was observed in the low frequency activity of the SN but for both animals the power in the 8–12 Hz and 13–25 Hz

frequency range was increased at the seizure onset. These initial increases of oscillatory activity in the SN were no longer significant at the end of the seizure for NHP 1 but remained significantly higher for NHP 2.

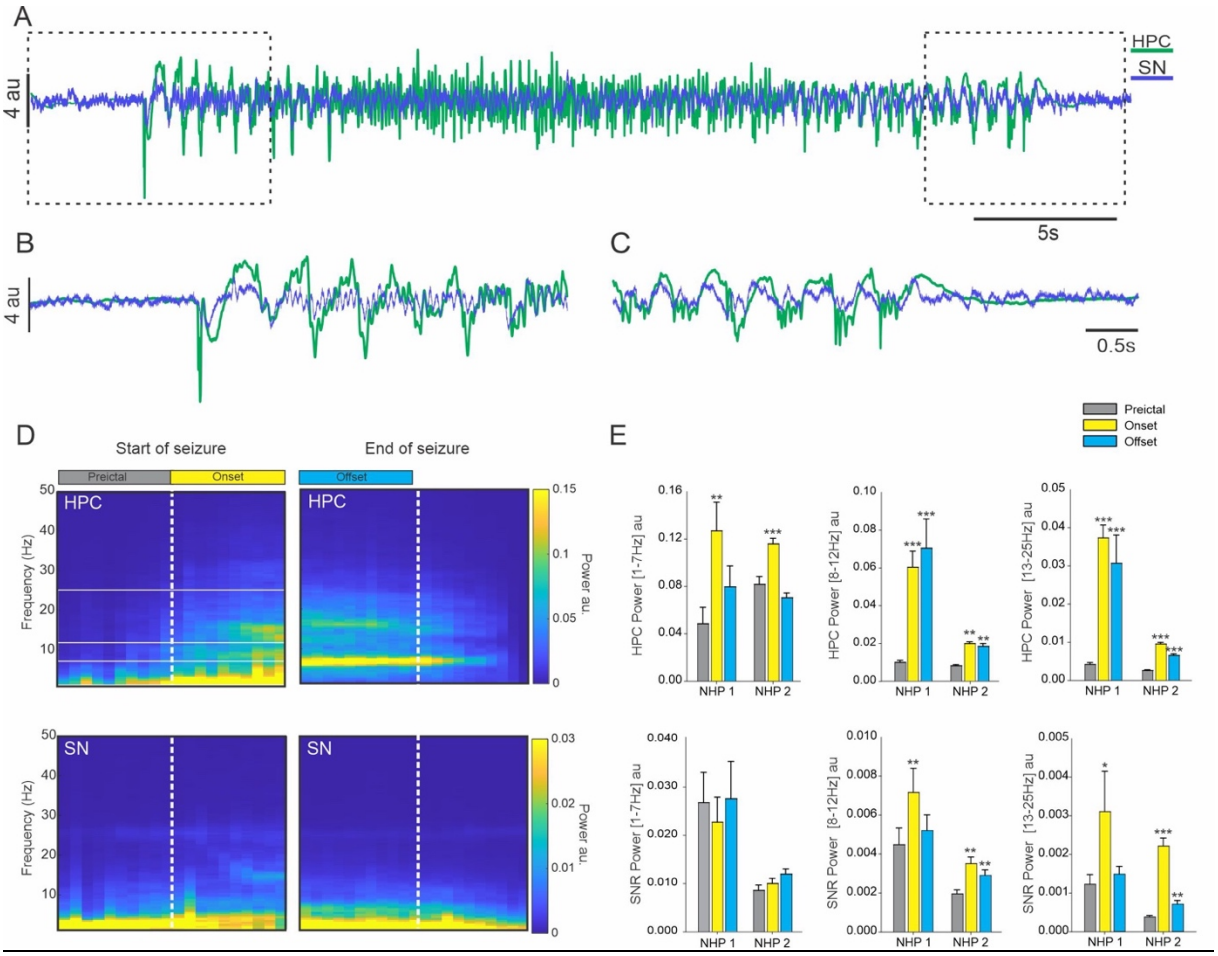

Figure 2: Power spectral density of the SN and HPC during seizures. (A) Example from NHP 1, of simultaneous recording obtained in the HPC and in the SN during a temporal lobe seizure with a focus on the onset (B) and offset (C) of the seizure. (D) Averaged spectral power obtained for NHP 1 in the HPC and SN at the beginning and end of the seizure. (E) Changes in the averaged power in the 1–7 Hz, 8–12 Hz and 13–25 Hz range in the HPC and SN for NHPs 1 and 2. Results are mean ± SEM. Statistical comparison performed with a Friedman repeated test and Dunnett's for post hoc comparison with the values preceding the seizures, *<0.05, **<0.01, ***<0.001.

3.2 Increase of coherence between the HPC and SN

For both animals, the increase in coherence between the HPC and SN was limited to the 13–25 Hz frequency band at the seizure onset (Figure 3). The coherence in the other frequency ranges examined was either decreased or unchanged (Supplementary Table 2).

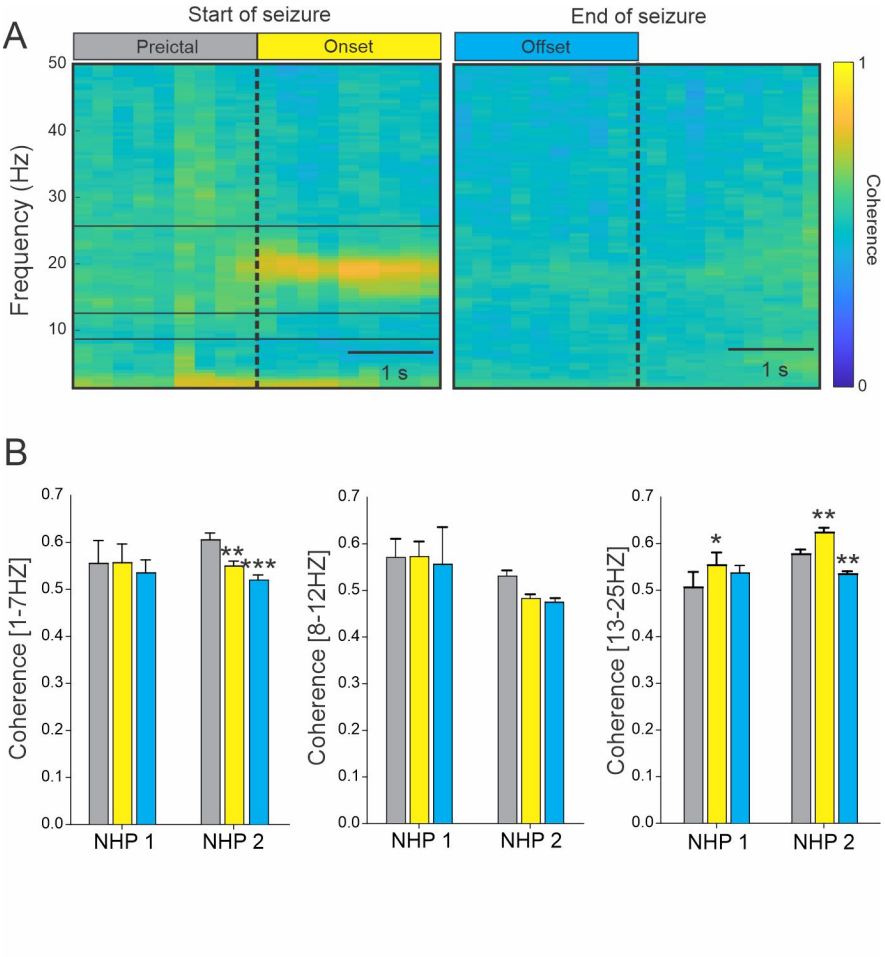

Figure 3: Coherence between HPC and SN during seizures. (A) Colormap representation of the averaged HPC/SN coherence obtained for NHP 2 at the beginning and end of the seizure. (B) The averaged HPC/SN coherence in the 13–25 Hz range for NHP 1 and 2. Results are mean ± SEM. Statistical comparisons were performed with a Friedman repeated test and Dunnett's for post hoc comparison with the values preceding the seizures, *<0.05, **<0.01.

3.3 SN recruitment is dependent of the seizure onset pattern

We identified a total of 18 seizures with an HAS onset pattern (22% and 25% of the seizures recorded in respectively NHP 1 and NHP 2) and 44 seizures with a LAF onset pattern (57% and 61% of the seizures recorded in NHP 1 and NHP 2). Eleven seizure onsets did not fall under either the HAS or LAS pattern and were therefore removed from subsequent analysis (21% and 14% respectively for NHP 1 and NHP 2). No difference was found in the proportion of seizure onset patterns between animals (Chi-square 0.22, Figure 4, A-C).

For both HAS and LAF onset seizures, the oscillatory power in the SN was increased in the 8–12 Hz and in the 13–25 Hz range (Figure 4D, Supplementary Table 3). The increase in HPC/SN coherence in the 13–25 Hz frequency range at the beginning of the seizure was specific to the LAF onset pattern (Figure 4E). No significant increase in HPC/SN coherence was found for the HAS onset pattern. However, we found a decrease of coherence in the 1–7 Hz frequency range and 13–25 Hz that was specific to the HAS onset pattern (Figure 4E, Supplementary Table 4). The HPC/SN coherence before the seizures was also greater for the HAS seizures compared to the LAF seizures in the 1–7 Hz and 13–25 Hz range (Figure 4E, Supplementary Table 4).

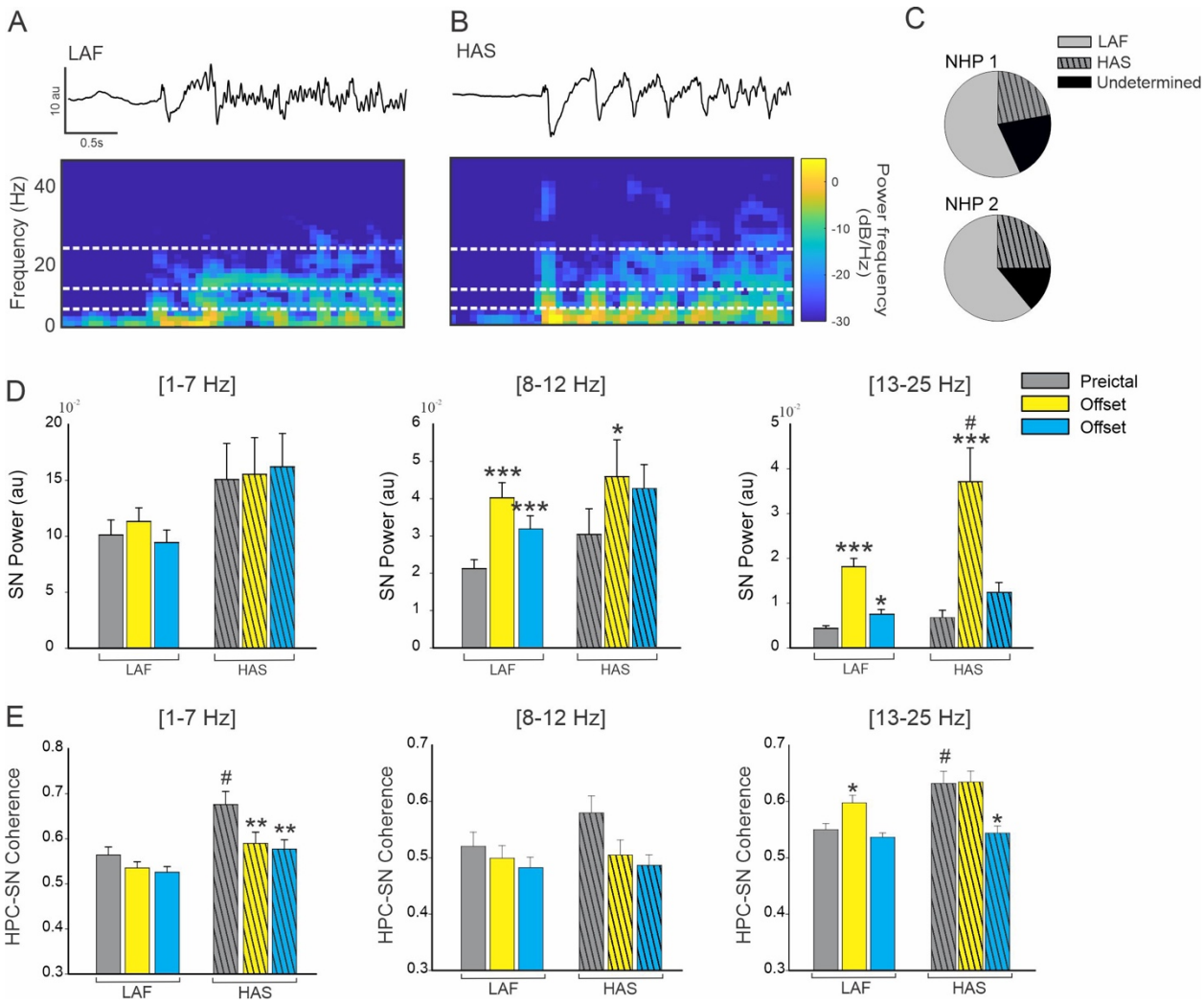

Figure 4: Effect of seizure onset pattern on HPC/SN activity during seizures. Example of typical LAF (A) and HAS (B) seizure showing HPC recording and corresponding spectral power. (C) Percentage of LAF, HAS, and undetermined seizures for NHP 1 and 2. (D) Changes in the averaged SN power and (E) HPC/SN coherence for LAF and HAS seizures. Results are mean ± SEM. Statistical comparison for each seizure onset pattern was performed with a Friedman repeated test and Dunnett's for post hoc comparison with the values preceding the seizures, *<0.05, **<0.01, ***<0.001. Comparisons between LAF and HAS seizures were performed with a Mann-Whitney Rank Sum test, # <0.05. Statistical values were corrected for multiple comparison using the Bonferroni method.

3.4 Cortical recruitment for HAS and LAF temporal seizures

In NHP 2, we recorded the activity of the SI during 15 HAS and 36 LAF seizures (Figure 5A). For the LAF seizures, we found an increase in cortical oscillatory power at the beginning of the seizures in the 1–7 Hz and 13–25 Hz ranges (Figure 5B). However, for both HAS and LAF seizures, the HPC/SI coherence in the 1–7 Hz range was increased at the beginning of the seizure. The cortical activity before and during HAS seizures was significantly lower than during LAF seizures (Figure 5B) however, the HPC/SI coherence values for HAS and LAF seizures were not significantly different (Supplementary Table 5).

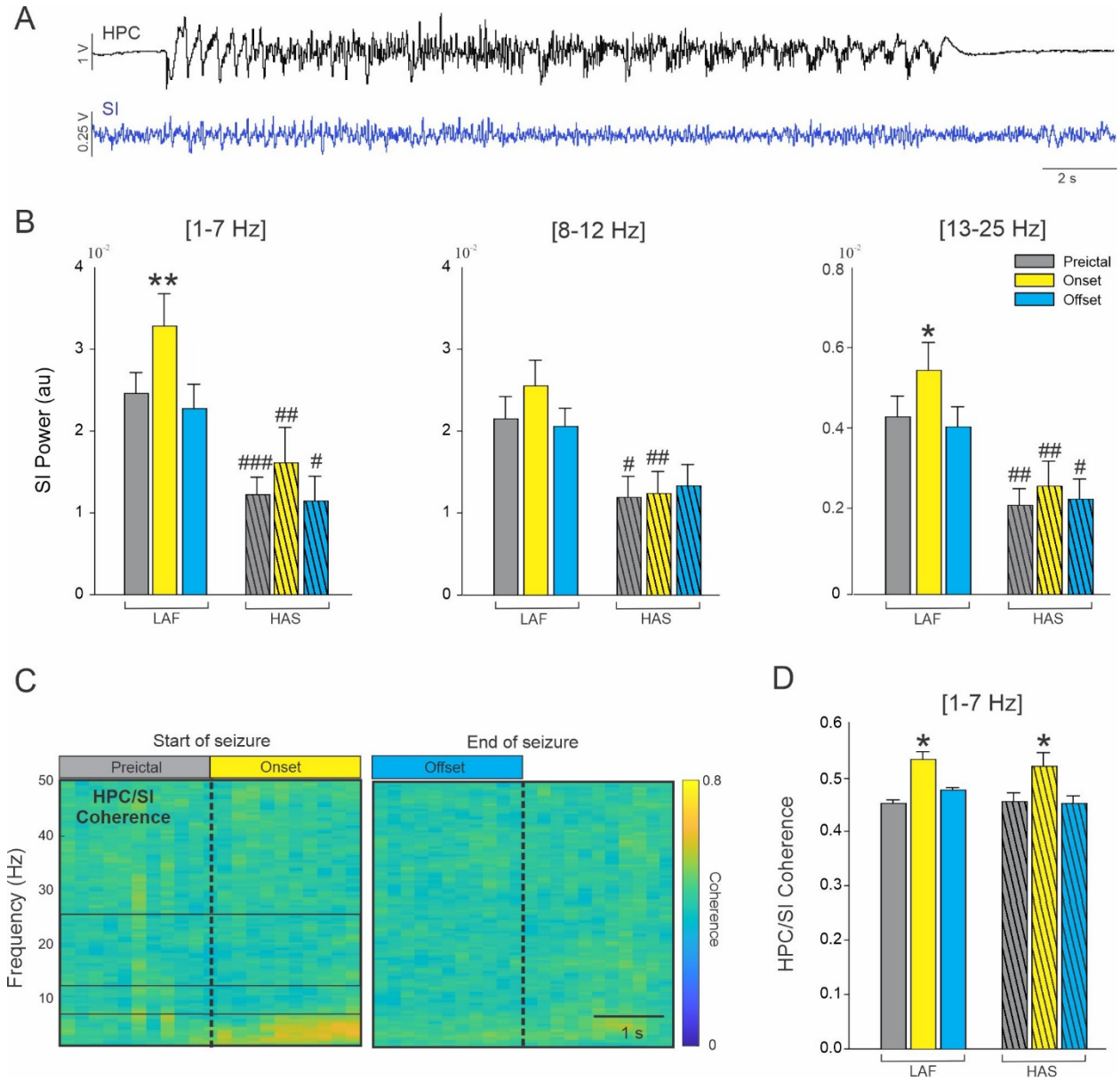

Figure 5: Influence of temporal lobe seizure onset pattern on cortical activity. (A) Example of simultaneous recordings obtained from the HPC and the SI in NHP 2. (B) Changes in the averaged SI power for LAF and HAS seizures. (C) Colormap of the averaged HPC/SI coherence (LAF and HAS combined). (D) Changes in the averaged HPC/SI coherence in the 1–7 Hz for LAF and HAS seizures. Results are mean ± SEM. Statistical comparisons were performed with a Friedman repeated test and Dunnett's for post hoc comparison with the values preceding the seizures, *<0.05, **<0.01. Comparisons between LAF and HAS seizures were

performed with a Mann-Whitney Rank Sum test, # <0.05, ##<0.01, ###<0.001. Statistical values were corrected for multiple comparison using the Bonferroni method.

4. Discussion

In this study we recorded LFP activity in the HPC, SN, and SI during PCN-induced temporal lobe seizures. We found that seizures with both LAF and HAS onset patterns were associated with an increase in HPC/SI coherence, while the increase in HPC/SN coherence was specific to the farther-spreading LAF onset pattern. This study is the first demonstration of temporal lobe seizures entraining the SN in the primate brain. Moreover, these findings provide evidence that this entrainment is dependent on the seizure onset pattern and support the hypothesis that the SN plays a role in the maintenance and generalization of temporal lobe seizures.

4.1 The role of the SN in the control of seizures

Three decades of rodent studies have established the anti-seizure effects of inhibiting the SN. In several models, inhibition of the SN using electrical and chemical interventions attenuated myoclonic movement, automatisms, and tonic-clonic convulsions [15, 17, 33] and non-convulsive seizures [20, 21]. There is also a long history of clinical data implicating the BG in temporal and extratemporal seizures using electrophysiological recordings [8-10], measuring cerebral metabolism [34, 35], and advanced fMRI analysis techniques [36]. However, to our knowledge, entrainment of the SN during a temporal lobe seizure has never been observed in the primate brain. Our finding that the coherence between the HPC and the SN is increased during the onset of temporal lobe seizures provides evidence that TL seizures implicate the primate SN as in rodents and suggest that modulation of the primate SN may have similar anti-seizure effects.

4.2 Entrainment of the SN is dependent on seizure onset pattern

Because of their importance in understanding ictogenesis and predicting treatment outcome, seizure onset patterns have recently drawn considerable attention [30, 37, 38]. The two most common onset patterns that are reported in patients with temporal lobe epilepsy are LAF and HAS [23, 39]. Similar to what has been described in clinical studies, our NHP model exhibited a prevalence of LAF onset pattern [23, 38, 39]. We found that both the LAF and HAS entrained the SI, but only LAF caused an increase in HPC/SN coherence. This finding corroborates clinical studies showing that LAF seizures are typically associated with a larger seizure onset zone and spread farther than HAS seizures in both temporal and extratemporal lobe seizures [23-27]. That only the further-spreading LAF seizures influenced the SN would support the theory that the SN plays a role in the propagation and generalization of seizures.

4.3 Propagation network and cortical entrainment of temporal lobe seizures

Multiple studies have shown the propagation of temporal lobe seizures to the SN. However, the pathway, mechanism, and role of the BG in temporal lobe seizures are still unclear. It has been suggested that BG activity is modulated by seizures via cortical areas [8, 40], and that the HPC-cortico-striatal connection is the entrance pathway leading to the entrainment of the BG. In support of this theory, studies have shown a preventive effect of GABA antagonist and dopamine agonist injections in the striatum [41-43]. Our results also agree with this theory of cortical recruitment, as the LAF seizure that were associated with an increase in HPC/SN coherence were also associated with an increase in HPC/SI coherence.

4.4 Limitations.

These results demonstrate the implication of the BG in temporal lobe epilepsy and the relevance of the seizure patterns when studying pathways and mechanisms. However, as is

often the case in NHP studies, the relatively small number of animals is a limitation. Additionally, while we did not observe obvious motor manifestations, we cannot exclude the possibility of changes in muscle contraction during temporal seizures as might be expected with propagation to the SI and SN. Along this line, we acknowledge that without comprehensive EEG recordings, we cannot conclude that the entrainment of the SN was mediated through cortical recruitment and it is possible that the entrainment of the SN could also be due to the seizure propagation and that the SN may not necessarily be involved in the epileptogenic network, per se [44]. To limit neural tissue damage caused by acutely targeting deep structures, we limited our recordings to one SN location. Therefore, we were unable to characterize any regional variability in SN seizure activity. In our study, the recordings were obtained from the posterior part of the SN on both animals, and it is well known that the topographical segregation of efferent and afferent connections in the SN is even more pronounced in the NHP than in the rodent [45]. Future studies can address this by recording and modulating different parts of the SN to evaluate the potential topography of seizure regulation in the NHP. In our study, we limited our analysis to HAS and LAF patterns, however, a more detailed distinction of the seizure patterns could be made [25].

5. Conclusion

This work demonstrates the implication of the SN in temporal lobe seizures. In addition, we demonstrated that the SN and cortex entrainment were dependent on the seizure onset pattern. These results confirm the idea that seizures arising from the same focus might have different mechanisms and might involve different pathways depending on their onset pattern. Finally, this study re-opens new lines of research into the control of temporal lobe seizures outside of the limbic network.

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