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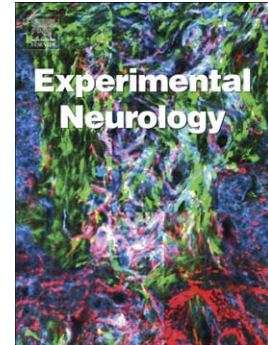
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## Pre-ictal increase in theta synchrony between the hippocampus and prefrontal cortex in a rat model of temporal lobe epilepsy

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### Abstract

The pathologically synchronised neuronal activity in temporal lobe epilepsy (TLE) can be triggered by network events that were once normal. Under normal conditions, hippocampus and medial prefrontal cortex (mPFC) work in synchrony during a variety of cognitive states. Abnormal changes in this circuit may aid to seizure onset and also help to explain the high association of TLE with mood disorders. We used a TLE rat model generated by perforant path (PP) stimulation to understand whether synchrony between dorsal hippocampal and mPFC networks is altered shortly before a seizure episode. We recorded hippocampal and mPFC local field potentials (LFP) of animals with spontaneous recurrent seizures (SRS) to verify the connectivity between these regions. We showed that SRS decrease hippocampal theta oscillations whereas coherence in theta increases over time prior to seizure onset. This increase in synchrony is accompanied by a stronger coupling between hippocampal theta and mPFC gamma oscillation. Finally, using Granger causality we showed that hippocampus/mPFC synchrony increases in the pre-ictal phase and this increase is likely to be caused by hippocampal networks. The dorsal hippocampus is not directly connected to the mPFC; however, the functional coupling in theta between these two structures rises pre-ictally. Our data indicates that the increase in synchrony between dorsal hippocampus and mPFC may be predictive of seizures and may help to elucidate the network mechanisms that lead to seizure generation.

Key-words: temporal lobe epilepsy, perforant path electrical stimulation, theta synchrony, ictal patterns, neurophysiology.

### Introduction

In temporal lobe epilepsy (TLE), the mechanisms underlying seizure development and the relationship between brain activity and seizure manifestation are yet poorly understood (Colom et al., 2006). In the epileptic brain, the synchronization becomes pathological thus generating seizures and several patterns of inter-ictal activity. The cognitive functions, including perception, formation and recall of memories, depend on integrated, often synchronous, activity of many neurons (Holmes, 2015; Jefferys et al., 2012; O'Neill et al., 2013). The limbic system, in general, and the hippocampal formation, in particular, has well-developed anatomical and physiological mechanisms that promote neuronal synchronization ripples (Jefferys et al., 2012). Examples of physiological synchronization include the theta rhythm, beta and gamma oscillations, and sharp-wave ripples (Jefferys et al., 2012). Human functional magnetic resonance imaging has shown that patients affected by TLE showed altered connectivity between the hippocampus and the medial prefrontal cortex (mPFC) (Kemmons et al., 2014, 2013). Rats with a history of seizures have deficits in synchronization across the hippocampus and mPFC, as measured by spectral coherence (Kleen et al., 2011). These studies suggest that seizure occurrence in TLE may contribute to abnormal hippocampal-mPFC connectivity.

The dorsal hippocampus does not project directly to the mPFC (Dégenétais et al., 2003). Nevertheless, these two structures show synchrony in theta oscillations during a variety of memory tasks (O'Neill et al., 2013). Theta oscillations generated in the dorsal hippocampus can reach the mPFC through the ventral hippocampus (O'Neill et al., 2013). mPFC theta has been associated with trace conditioning, memory and attentional set-shifting (O'Neill et al., 2013; Paz et al., 2008). Besides, theta oscillations in mice mPFC have also arisen during anxiety-like behaviour (Adhikari et al., 2010). Hippocampal neurons projecting to the mPFC arise mostly from the ventral CA1 and subiculum through the fimbria/fornix (Jay and Witter, 1991). There is no described connection arising from the mPFC onto the hippocampus (Jay and Witter, 1991; O'Neill et al., 2013); hence, the connection between the dorsal hippocampus and the mPFC is multisynaptic (especially through the ventral CA1/subiculum) (Adhikari et al., 2010; Jay and Witter, 1991; O'Neill et al., 2013).

Rats exposed to early-life seizures showed a markedly higher coherence between the dorsal and ventral hippocampus and the mPFC in theta and several other frequency bands (Holmes, 2015). Besides, there is a remarked increase in activity synchrony between the dorsal hippocampus and the mPFC in schizophrenia models (Dickerson et al., 2010). Increased synchrony between hippocampus and mPFC in epilepsy could alter cortical networks, helping to explain the association between TLE and mood disorders. However, synchrony between hippocampus and mPFC during pre-ictal and post-ictal activity has not been systematically studied.

In TLE, recurrent simple or complex partial seizures initiate in the temporal lobe. In this disorder, seizures rarely become generalized, and there is a marked hippocampal sclerosis (Sloviter, 2005). There is also evidence that oscillatory processes may trigger seizures (Nazer and Dickson, 2009). For example, it has been reported a correlation between the amplitude of slow wave oscillations and epileptiform discharges (Nazer and Dickson, 2009). The link between physiological oscillatory activity and epilepsy is still obscure but higher excitation states observed in low-frequency oscillations may participate in seizure initiation (Nazer and Dickson, 2009). Theta oscillations appear to be particularly relevant to seizure initiation. Head-

fixed recordings from rabbits (kindling model) showed an increase in hippocampal theta rhythm before spontaneous recurrent seizures (SRS) (Kitchigina and Butuzova, 2009). Long epochs of theta oscillation were also observed in recordings from pilocarpine-treated rats (Grasse et al., 2013). Lévesque and others (2013) also showed increased theta oscillations in the hippocampus CA3 and entorhinal cortex before epileptiform discharges induced by 4-aminopyridine. In a rat model of absence epilepsy, mPFC EEG segments preceding spontaneous discharges showed a remarked increase in theta at moments preceding epileptiform discharges (Sitnikova and van Luijtelaaar, 2009).

Most TLE animal models involve the induction of prolonged status epilepticus (Sloviter, 2005). However, these models show several features uncommon to TLE in humans like severe brain damage, behaviour abnormality and high incidence of generalised seizures (Sloviter, 2005). The TLE model generated by perforant path (PP) stimulation produces moderate hippocampal sclerosis and pathophysiological changes similar to changes found in patients with TLE (Harvey and Sloviter, 2005). Due to stimulation at moderate intensities, aberrant activity is restricted to the epileptic circuitry causing relatively focal tissue damage (Sloviter et al., 2007). Hippocampus afterdischarges can be induced without the complications of direct excitotoxic damage, prominent temporal and extratemporal damage, that results from the kainic acid or pilocarpine models (Kandratavicius et al., 2014; Reddy and Kuruba, 2013). Hence, the PP stimulation model is ideal for studying the effect of seizures in the connectivity of downstream circuits (that present milder alterations during epileptogenesis and TLE chronic phase).

The identification of ictal patterns relating to clinical features is relevant to the generation of animal models used to validate pathological changes in epilepsy and mainly for the investigation of paroxysmal abnormalities that are ictal markers. Hence, TLE animal models can help to elucidate epileptic focus areas and to predict the occurrence of seizures or post-ictal neurological changes. Such tools have important clinical relevance for understanding seizures generating mechanisms, as well as for ictal treatment and extinction. In this work, using the TLE model generated by PP stimulation, we recorded hippocampal and mPFC local field potentials (LFP) of animals with SRS to understand whether synchrony between these two structures is altered shortly before a seizure episode. We found that coherence at theta frequency (6-9Hz) increased in the pre-ictal period preceding a seizure. Also, using Granger causality, we show that hippocampus theta strongly influences mPFC theta.

## **Material and Methods**

### *Subjects*

Experiments were performed on adult male Wistar rats weighing 280-350 g. Rats were housed in standard rodent cages in a colony room maintained at 24°C under a 12 h light-12 h dark cycle with free access to food and water. All procedures were performed according to the Brazilian Council for the Control of Animal Experimentation (CONCEA) guidelines for animal research and approved by the ethics committee at the University of São Paulo. These guidelines abide by the National Institute of Health rules for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Experiments were designed to minimize the number of animals used and their suffering.

### *Surgery and electrodes implants*

Rats were anesthetized with ketamine (0.5 mg/Kg, i.p. and 0.75 mg/Kg i.m.) and xylazine (0.25 mg/Kg, i.p. e 0.38 mg/kg, i.m.) and placed in a stereotaxic frame for electrode implantation. Body temperature was kept at  $37\pm 0.5^{\circ}\text{C}$  by a heating pad (Insight, Brazil). The level of anesthesia was constantly checked by tail pinch reflex, respiratory rate and EEG and we applied supplementary doses of the anaesthetic (10% of the initial dose) when necessary. After the skull was exposed, we drilled ipsilateral holes for accessing angular bundles of the PP (anteroposterior, A.P: - 7.6 mm; lateral to midline, L: - 4.5 mm; ventral to dura mater, V: - 2.8 mm), dentate gyrus (DG; A.P: - 3.3 mm, L: - 2.2 mm, V: - 3.0 mm) and mPFC, (A.P: + 3.0 mm, L: - 0.5 mm; V: - 3.2 mm) (Paxinos and Watson, 2007). Silver wires soldered to micro-screws served for stimulus ground and recording ground/reference electrodes (placed respectively at the contralateral parietal cortex and cerebellum). We stimulated the PP and recorded from DG and mPFC using teflon-insulated tungsten wires (60 $\mu\text{m}$  diameter). We optimised the position of DG electrodes by monitoring population spikes in the DG, and we fixed the electrodes using dental cement. The animals then received a subcutaneous injection of flunixin meglumine (Banamine, 2.5 mg/kg) and were returned to their cages and to recover for at least seven days.

### *PP intermittent and long-term stimulation*

After a week of recovery from surgery, rats were placed in the experimental box for PP stimulation and LFP recording. We initially plotted input-output functions for each subject to calculate the minimum intensity necessary to induce afterdischarges in the DG avoiding SE induction. Monophasic test pulses (200 ms duration) were delivered every 20s at increasing intensities (70-700  $\mu\text{A}$ ), and the resulting fPSPs were used to compute input-output curves (S88 stimulator; Grass Instruments Co.). Long-term electrical stimulation pulse was set based on the generation of three consecutive population spikes of similar amplitude. PP electrical stimulation in freely moving animals consisted of 10-s trains of 20-Hz stimuli (200 1ms-monophasic pulses) with 50s intervals during 8h (480 stimulus trains) (Figure 1B-C) (Sloviter et al., 2007). fPSPs and LFP were pre-amplified and band-pass filtering (gain=100x, 0.03-3kHz; P55-AC pre-amplifier, Grass Instruments Co.) and digitized at 10 kHz (PowerLab/16S; LabChart-AD Instruments).

### *Data analysis*

We visually inspected LFP recordings for SRS occurrence. Seizure onset was marked by the first spike (time at 5% of the peak spike amplitude) before the flattening of the EEG that precedes the paroxysmal activity (Queiroz et al., 2009). The end of the paroxysmal activity was characterized by its disappearance, by depression of the EEG amplitude and occasionally by low-frequency post-ictal bursts (Queiroz et al., 2009) (Figure 1-D). Once seizures were detected, we analyzed 120s LFP segments pre and post-ictally. Pre and post-ictal recordings with signal artifacts (events with amplitude  $> 2.5x$  the standard deviation of the segment) were excluded from analysis. Theta power was presented as theta/delta ratio (Buzsáki et al., 2003; Csicsvari et al., 1998). Seizures that occurred within 20 minutes of a preceding seizure were also excluded from analysis, to avoid post-ictal effects in pre-ictal

activities patterns. Inter-ictal recordings were obtained from hippocampus and mPFC LFP in basal state (i.e. not associated to seizures events). In order to compare increased pre-ictal coherence with inter-ictal theta, we extract randomly, 120s LFP epochs from 2 months-recordings. Only LFP epochs with a maximum ratio of theta peak/delta peak were used of each animal, which ensured the use of LFP with theta activity. Power spectral densities (PSD) of DG and mPFC LFP selected segments were calculated for epochs of 30 or 120s with the Matlab *pwelch* function (Mathworks, Inc., Natick, USA), using a 6000 points Hanning window with 50% overlap. Signal power at 58-62 Hz was excluded from the spectra due to 60 Hz-noise contamination. Time-frequency power spectrum calculation was performed using the *spectrogram* function with the same parameters of PSD analysis. Coherence between the two recording sites was calculated using the *mscohere* function, with 4000 points window and 75% overlap. Theta amplitude envelope was calculated from LFP filtered at theta-band using the *eegfilt* command from the EEGLAB toolbox (<http://sccn.ucsd.edu/eeglab/>) followed by Hilbert transform (Matlab *hilbert* function). Phase-amplitude cross-frequency coupling was computed using the modulation index (Tort et al., 2010). For the comodulation maps, we bandpass filtered signals using 10-Hz windows and 5-Hz steps for the amplitude frequencies, and 2-Hz windows at 0.5-Hz steps for the phase frequencies. For computing the mean theta-gamma modulation index, we filtered LFP recorded from electrodes in DG between 3-8 Hz for theta, and from mPFC in the interval of 30-80 Hz for gamma. Spectral Granger causality was computed according to Barnett and Seth (2014) using a freely available Matlab toolbox ([http://users.sussex.ac.uk/~anils/aks\\_code.htm](http://users.sussex.ac.uk/~anils/aks_code.htm)). Unless otherwise noted, statistical tests were performed using paired t-test (Matlab *ttest* function).

## Results

This study aimed to assess changes in mPFC/dorsal hippocampus synchrony immediately preceding a seizure episode. Intermittent long-term stimulation was performed in 21 rats, although only 11 rats developed SRS. A total of 31 seizures from 11 rats were used in this study. The majority of seizures occurred during sleep (66.6%, these rats were sleeping at least 3 minutes before seizure onset), 12.5% of animals were exploring 30s before the seizure, and 20.83% were awake but immobile. Hippocampus and mPFC LFP were recorded from chronically implanted electrodes in these two regions. Ictal activity initiated at the hippocampus in all seizures and propagated to mPFC, with a mean delay of 3.29 s,  $\pm$  0.84s. Mean seizure duration was equal to 69.68s,  $\pm$  4.59s and mean latency from the induction protocol to the first SRS was 34.5,  $\pm$  6.7 days.

We first analyzed theta oscillations before and after a seizure event. Mean theta power of 120s segments of hippocampus LFP significantly decreased in the post-ictal phase when compared to pre-ictal theta (Figure 2A-C). Mean hippocampal theta power decreased from  $0.0037 \pm 0.0007$  to  $0.0012 \pm 0.0001$   $\text{mV}^2/\text{Hz}$  ( $p=0.0002$ ,  $n=11$  rats, Figure 2B-C). We found an overall drop in post-seizure LFP power in the hippocampus in the 30s post-ictal period followed by a significant increase 120s after seizure ( $0.0007 \pm 0.0002$  to  $0.0015 \pm 0.0002$   $\text{mV}^2/\text{Hz}$ ,  $p=0.003$ , Figure 2-C). However, no difference in theta power was observed in the mPFC when comparing pre and post-ictal periods (Figure 2B-C). Taken together, our data shows that SRS decrease hippocampal theta oscillations.

We next analyzed 30s epochs of the hippocampus and mPFC LFP, 120s and 30s preceding seizure episodes. Mean theta/delta power significantly increased in LFP recorded 120s to 30s in the pre-ictal period in the hippocampus (45% of increase,  $p=0.02$ ,  $n=31$  seizures, 11 rats, Figure 3). In animals with seizures, 81.8% (9 out of 11 animals) showed increase in hippocampus theta/delta power prior to seizure onset, and when all pre-seizure segments were pulled together, we found that theta/delta increased in 67% (21 out of 31 seizures) of the 30s pre-ictal segments. We then tested the hypothesis that coherence between hippocampus and mPFC increases at the moments preceding the seizure. We checked coherence in theta between mPFC and hippocampus changes in the pre and post-ictal periods. Mean theta coherence decreased significantly in the post-ictal period compared to coherence preceding seizures (from  $0.30\pm 0.03$  to  $0.16\pm 0.02$ ,  $p=0.00002$ ,  $n=31$  seizures, Figure 4A and 4C-D). We then analyzed whether coherence increased over time prior to seizure onset. We found a significant increase in theta coherence preceding seizures (Figure 4B-D). Mean theta coherence increased immediately preceding the seizure when compared to the coherence 120s in the pre-ictal period (from  $0.20\pm 0.04$  to  $0.30\pm 0.06$ ,  $p=0.0006$ ,  $n=31$  seizures, Figure 4B-D). The time course of the mean coherence increase is shown in Figure 4C. Theta coherence has also significantly dropped when seizures started (Figure 5A-C). The onset of seizures is also marked by an instantaneous drop in theta coherence (Figure 5B). Mean theta coherence decreased from  $0.30\pm 0.03$  to  $0.10\pm 0.03$ ,  $p=0.00005$ ,  $n=31$ , Figure 5C). Theta coherence between mPFC/hippocampus was also greater immediately before seizures in relation to theta coherence segments that seizure onset was shuffled ( $0.30\pm 0.03$  to  $0.17\pm 0.03$ ,  $p=0.02$ ,  $n=31$ ). Besides, coherence in theta in the 30s preceding seizures was significantly greater than theta coherence during inter-ictal theta ( $p=0.01$ ,  $n=31$ , Figure 5D). These results indicate that coherence in theta increases shortly before SRS.

We compared pre-ictal and inter-ictal (during theta oscillations unrelated to SRS) to assess whether the pre-ictal increase in theta coherence is a genuine marker of synchronization. Our purpose was to exclude the possibility of an effect of simultaneous oscillations of equal voltage at both electrodes produced by volume conduction of currents. We found little correlation between hippocampal theta amplitude (instantaneous amplitude calculated from the Hilbert transform of theta-filtered signals) and theta coherence in pre-ictal recordings (Figure 6A-C). However, during basal theta episodes, there was a strong correlation between hippocampal theta amplitude and theta coherence (mean pre-ictal correlation coefficient,  $r$  equal to  $0.13\pm 0.05$  vs.  $0.35\pm 0.05$  during inter-ictal theta epochs,  $p=0.003$ , Figure 6B-C). These results indicate that volume conduction does not cause the increase in functional connectivity between the hippocampus and mPFC preceding SRS. These findings also suggest that hippocampus-mPFC theta synchrony in the pre-ictal period occurs through a different mechanism than synchrony during basal theta.

It has been shown that hippocampal theta oscillations are coupled to mPFC theta and modulates gamma-band oscillations within the hippocampus during several behavioural tasks ( Siapas et al., 2005; Jones and Wilson, 2005; Tort et al., 2008). Hippocampal theta also modulates prefrontal gamma oscillations especially during REM sleep (Scheffzük et al., 2011). Hence, we hypothesised that mPFC gamma is coupled to pre-ictal theta at the moments preceding the seizure. We first analysed cross-frequency modulation within the hippocampus 30s and 120s in the pre-ictal period. Gamma (40-100Hz) increased significantly in LFP recorded 30s preceding

seizure episodes when compared to LFP from 120s before seizures (30s segments, Figure 7A and B). Means theta-gamma cross-frequency modulations index within the hippocampus were  $0.0015 \pm 0.00003$  and  $0.0023 \pm 0.00006$ , 30s and 120s before seizure events, respectively ( $p=0.03$ ,  $n=31$  seizures, Figure 7A and B). Prefrontal gamma amplitude modulation by hippocampal theta phase also increased preceding seizures. Theta-gamma modulation indexes were  $0.0013 \pm 0.00003$  and  $0.0021 \pm 0.00006$ , 120s and 30s before seizures, respectively ( $p=0.04$ ,  $n=31$  seizures, Figure 7C and D). We found no differences in gamma power between 120s and 30s recordings before seizures in both hippocampus and mPFC recordings. These results support the idea that in the pre-ictal period, hippocampal theta coordinates mPFC networks.

We further tested the hypothesis that hippocampal theta leads mPFC activity using Granger causality (Barnett and Seth, 2014). We calculated causality at each direction (hippocampus theta causing mPFC theta or mPFC theta causing hippocampus theta). Spectral Granger causality at each directions hippocampus  $\rightarrow$  mPFC and mPFC  $\rightarrow$  hippocampus was not significantly at theta frequencies 120s before seizures. Figure 8A-B shows an example of hippocampus and mPFC LFP filtered at theta band 120s and 30s before a seizure and the respective spectral Granger analysis considering causality between hippocampus and mPFC and vice-versa. Mean mPFC  $\rightarrow$  hippocampus and hippocampus  $\rightarrow$  mPFC Granger causality in theta was not significantly different 120s in the pre-ictal phase. However, 30s before seizures, hippocampus  $\rightarrow$  mPFC causality was significantly greater ( $p=0.02$ ,  $n=31$  seizures) than mPFC  $\rightarrow$  hippocampus at theta-band frequencies (Figure 8C and D). These results further support the idea that hippocampus/mPFC synchrony increases in the pre-ictal period and this increase is likely to be caused by hippocampal networks.

## Discussion

Changes in brain oscillations in the inter-ictal period provide information about the epileptogenic network and potential ictal sources. Besides, pre-ictal changes in the LFP may be important tools in the study of predicting crisis. We have used a rat TLE model to show that synchrony (at theta frequency) between the hippocampus and mPFC increases in periods preceding seizures. We first showed that hippocampus and mPFC coherence increases monotonically in the pre-ictal phase, specifically in theta frequency. This increase was accompanied by theta/delta power rise in hippocampus but with little correlation between theta amplitude and coherence and no difference in delta coherence. We then demonstrated that hippocampal theta also modulates prefrontal gamma oscillations before seizures and, using Granger causality, we showed that the increase in theta coherence is likely to have arisen from the hippocampus.

Pre-seizure LFP analysis in PP intermittent stimulation model showed that theta power increases preceding seizures. This result corroborates with previous reports that show a pre-ictal increase in hippocampal theta in different animal models (Grasse et al., 2013; Kitchigina and Butuzova, 2009; Sedigh-Sarvestani et al., 2014). While we report a pre-ictal increase in theta coherence between the hippocampus and mPFC, there are several studies that show a reduction of physiological theta in the inter-ictal periods in TLE models. Disruption of exploratory theta was reported during the inter-ictal phase in TLE experimental models and humans (Arabadzisz et al., 2005; Bettus et al., 2008; Chauvière et al., 2009; Inostroza et al., 2013; Laurent et al.,



2015). A study using kainic acid injections as a TLE model showed that theta oscillations are lower during latent and chronic phases compared to basal theta (Arabadzisz et al., 2005). In TLE patients, spectral analysis of intracranial EEG recordings showed a marked reduction in theta during inter-ictal periods (Bettus et al., 2008). Theta power is also reduced after SE, and this reduction has been correlated to deficits in hippocampus-dependent spatial memory tasks (Chauvière et al., 2009).

Theta and gamma oscillations are normally present in the hippocampus and prefrontal cortex during various cognitive tasks (O'Neill et al., 2013; Tort et al., 2008). While not being directly connected to the mPFC (Hoover and Vertes, 2007), theta in the dorsal hippocampus can modulate mPFC spiking (O'Neill et al., 2013). The ventral hippocampus, however, is monosynaptically connected to the mPFC (Hoover and Vertes, 2007). Ventral hippocampus theta coincides in both frequency and power with mPFC theta oscillations during spatial working memory tasks and anxiety-like behaviour (Adhikari et al., 2010; O'Neill et al., 2013). In the non-match-to-sample T-maze test, dorsal hippocampus and mPFC theta coherence increases during the 'choice phase' (O'Neill et al., 2013). Our Granger causality computations imply that the dorsal hippocampus plays a leading role in the increase in coherence in mPFC. The lack of direct connection suggests, therefore, that coherence increase between the dorsal hippocampus and the mPFC may be relayed by the ventral hippocampus or thalamus (O'Neill et al., 2013). Thus, the increase in theta coherence between the dorsal and ventral hippocampus could be explained by an increase dorsal/ventral hippocampus synchrony initiated by the dorsal hippocampus (Patel et al., 2012). It will be interesting in the future to record simultaneously pre-ictal LFP from dorsal and ventral hippocampi and the mPFC.

The increase pre-ictal coherence between hippocampus and mPFC could arise from volume conduction (Scheffzük et al., 2011). However, the correlation between hippocampal theta amplitude and theta coherence was significantly lower in pre-ictal recordings, suggesting that the observed coherence increase was not associated with volume conduction. Also, we found that gamma oscillations in the mPFC are significantly modulated 30s preceding seizures (but not at 120s). While slow oscillations in the LFP may arise from distant locations, gamma oscillations are believed to be generated locally (Sirota et al., 2008). Hence, the modulation of gamma amplitude in the mPFC by hippocampal theta phase cannot be explained by volume conduction but rather by a genuine influence of hippocampal activity in mPFC circuits (Bitzenhofer et al., 2015; Harris and Gordon, 2015). In addition, the change in spectral Granger causality preceding seizures (indicating a leading role of hippocampal networks) is a strong indicative that the increase in coherence arises from a genuine change in pre-ictal functional connectivity between the hippocampus and the mPFC (Elsegai et al., 2015).

We have found that hippocampal theta modulates gamma oscillations strongly at moments preceding seizures. The increased top-down influence of the dorsal hippocampus in the mPFC preceding seizures indicates that the transient increase in functional connectivity between these two structures may be mechanistically related to the seizure onset. A similar increase in hippocampal theta phase/parietal cortex gamma was observed during REM sleep (Scheffzük et al., 2011). Also, hippocampal theta-mPFC gamma coupling has been thought to induce plasticity in mPFC circuits (Zheng and Zhang, 2015). Abnormal plasticity in TLE could, therefore, be associated

with cellular changes occurring in the mPFC (Tang and Loke, 2010). Deficits in hippocampus-mPFC synchrony are associated with psychosis and mood disorders (Lee et al., 2014; Zheng and Zhang, 2015). The scope of this paper was to investigate if functional hippocampal/mPFC connectivity precedes ictal events and mechanistically associate with seizure generation. However, future experiments with specific behavioural tasks will clarify if hippocampal/mPFC connectivity altered in epileptic rats, as presented in this study, could help to explain the strong link between epilepsy and mood disorders.

In summary, we have shown that theta band synchrony between the dorsal hippocampus and mPFC increases immediately prior to seizure onset. This increase in synchrony is accompanied by a stronger coupling between hippocampal theta and mPFC gamma oscillation. Besides, we have shown that the increase in synchrony is followed by a hippocampus to mPFC directionality. Taken together, our data indicates that the increase synchrony between dorsal hippocampus and mPFC may be predictive of seizures. Also, the hippocampal theta-mPFC gamma modulation, similar to cross-frequency modulation occurring during specific cognitive states, may help to elucidate the network mechanisms that lead to the generation of seizures.

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### References

- Adhikari, A., Topiwala, M.A., Gordon, J.A., 2010. Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. *Neuron* 65, 257–269. doi:10.1016/j.neuron.2009.12.002
- Arabadzisz, D., Antal, K., Parpan, F., Emri, Z., Fritschy, J.-M., 2005. Epileptogenesis and chronic seizures in a mouse model of temporal lobe epilepsy are associated with distinct EEG patterns and selective neurochemical alterations in the contralateral hippocampus. *Exp. Neurol.* 194, 76–90. doi:10.1016/j.expneurol.2005.01.029
- Barnett, L., Seth, A.K., 2014. The MVGC multivariate Granger causality toolbox: a new approach to Granger-causal inference. *J. Neurosci. Methods* 223, 50–68. doi:10.1016/j.jneumeth.2013.10.018
- Bettus, G., Wendling, F., Guye, M., Valton, L., Régis, J., Chauvel, P., Bartolomei, F., 2008. Enhanced EEG functional connectivity in mesial temporal lobe epilepsy. *Epilepsy Res.* 81, 58–68. doi:10.1016/j.eplesyres.2008.04.020
- Bitzenhofer, S.H., Sieben, K., Siebert, K.D., Spehr, M., Hanganu-Opatz, I.L., 2015. Oscillatory activity in developing prefrontal networks results from theta-gamma-modulated synaptic inputs. *Cell Rep.* 11, 486–497. doi:10.1016/j.celrep.2015.03.031

- Buzsáki, G., Buhl, D.L., Harris, K.D., Csicsvari, J., Czéh, B., Morozov, A., 2003. Hippocampal network patterns of activity in the mouse. *Neuroscience* 116, 201–211.
- Chauvière, L., Raftafi, N., Thinus-Blanc, C., Bartolomei, F., Esclapez, M., Bernard, C., 2009. Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. *J. Neurosci. Off. J. Soc. Neurosci.* 29, 5402–5410. doi:10.1523/JNEUROSCI.4699-08.2009
- Colom, L.V., García-Hernández, A., Castañeda, M.T., Perez-Cordova, M.G., Garrido-Sanabria, E.R., 2006. Septo-Hippocampal Networks in Chronically Epileptic Rats: Potential Antiepileptic Effects of Theta Rhythm Generation. *J. Neurophysiol.* 95, 3645–3653. doi:10.1152/jn.00040.2006
- Csicsvari, J., Hirase, H., Czurko, A., Buzsáki, G., 1998. Reliability and state dependence of pyramidal cell-interneuron synapses in the hippocampus: an ensemble approach in the behaving rat. *Neuron* 21, 179–189.
- Dégenétais, E., Thierry, A.-M., Glowinski, J., Gioanni, Y., 2003. Synaptic influence of hippocampus on pyramidal cells of the rat prefrontal cortex: an in vivo intracellular recording study. *Cereb. Cortex N. Y. N* 1991 13, 782–792.
- Dickerson, D.D., Wolff, A.R., Bilkey, D.K., 2010. Abnormal long-range neural synchrony in a maternal immune activation animal model of schizophrenia. *J. Neurosci. Off. J. Soc. Neurosci.* 30, 12424–12431. doi:10.1523/JNEUROSCI.3046-10.2010
- Elsegai, H., Shiells, H., Thiel, M., Schelter, B., 2015. Network inference in the presence of latent confounders: the role of instantaneous causalities. *J. Neurosci. Methods* 245, 91–106. doi:10.1016/j.jneumeth.2015.02.015
- Grasse, D.W., Karunakaran, S., Moxon, K.A., 2013. Neuronal synchrony and the transition to spontaneous seizures. *Exp. Neurol.* 248, 72–84. doi:10.1016/j.expneurol.2013.05.004
- Harris, A.Z., Gordon, J.A., 2015. Long-Range Neural Synchrony in Behavior. *Annu. Rev. Neurosci.* 38, 171–194. doi:10.1146/annurev-neuro-071714-034111
- Harvey, B.D., Sloviter, R.S., 2005. Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. *J. Comp. Neurol.* 488, 442–463. doi:10.1002/cne.20594
- Holmes, G.L., 2015. Cognitive impairment in epilepsy: the role of network abnormalities. *Epileptic Disord. Int. Epilepsy J. Videotape* 17, 101–116. doi:10.1684/epd.2015.0739
- Hoover, W.B., Vertes, R.P., 2007. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct. Funct.* 212, 149–179. doi:10.1007/s00429-007-0150-4
- Inostroza, M., Brotons-Mas, J.R., Laurent, F., Cid, E., de la Prida, L.M., 2013. Specific impairment of “what-where-when” episodic-like memory in experimental models of temporal lobe epilepsy. *J. Neurosci. Off. J. Soc. Neurosci.* 33, 17749–17762. doi:10.1523/JNEUROSCI.0957-13.2013
- Jay, T.M., Witter, M.P., 1991. Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *J. Comp. Neurol.* 313, 574–586. doi:10.1002/cne.903130404
- Jefferys, J.G.R., Jiruska, P., de Curtis, M., Avoli, M., 2012. Limbic Network Synchronization and Temporal Lobe Epilepsy, in: Noebels, J.L., Avoli, M., Rogawski, M.A., Olsen, R.W., Delgado-Escueta, A.V. (Eds.), *Jasper’s Basic*

- Mechanisms of the Epilepsies. National Center for Biotechnology Information (US), Bethesda (MD).
- Kandratavicius, L., Balista, P.A., Lopes-Aguiar, C., Ruggiero, R.N., Umeoka, E.H., Garcia-Cairasco, N., Bueno-Junior, L.S., Leite, J.P., 2014. Animal models of epilepsy: use and limitations. *Neuropsychiatr. Dis. Treat.* 10, 1693–1705. doi:10.2147/NDT.S50371
- Kemmotsu, N., Kucukboyaci, N.E., Cheng, C.E., Girard, H.M., Tecoma, E.S., Iragui, V.J., McDonald, C.R., 2013. Alterations in functional connectivity between the hippocampus and prefrontal cortex as a correlate of depressive symptoms in temporal lobe epilepsy. *Epilepsy Behav.* EB 29, 552–559. doi:10.1016/j.yebeh.2013.09.039
- Kemmotsu, N., Kucukboyaci, N.E., Leyden, K.M., Cheng, C.E., Girard, H.M., Iragui, V.J., Tecoma, E.S., McDonald, C.R., 2014. Frontolimbic brain networks predict depressive symptoms in temporal lobe epilepsy. *Epilepsy Res.* 108, 1554–1563. doi:10.1016/j.epilepsyres.2014.08.018
- Kitchigina, V.F., Butuzova, M.V., 2009. Theta activity of septal neurons during different epileptic phases: the same frequency but different significance? *Exp. Neurol.* 216, 449–458. doi:10.1016/j.expneurol.2009.01.001
- Kleen, J.K., Wu, E.X., Holmes, G.L., Scott, R.C., Lenck-Santini, P.-P., 2011. Enhanced oscillatory activity in the hippocampal-prefrontal network is related to short-term memory function after early-life seizures. *J. Neurosci. Off. J. Soc. Neurosci.* 31, 15397–15406. doi:10.1523/JNEUROSCI.2196-11.2011
- Laurent, F., Brotons-Mas, J.R., Cid, E., Lopez-Pigozzi, D., Valero, M., Gal, B., de la Prida, L.M., 2015. Proximodistal structure of theta coordination in the dorsal hippocampus of epileptic rats. *J. Neurosci. Off. J. Soc. Neurosci.* 35, 4760–4775. doi:10.1523/JNEUROSCI.4297-14.2015
- Lee, H., Dvorak, D., Fenton, A.A., 2014. Targeting Neural Synchrony Deficits is Sufficient to Improve Cognition in a Schizophrenia-Related Neurodevelopmental Model. *Front. Psychiatry* 5, 15. doi:10.3389/fpsyt.2014.00015
- Lévesque, M., Salami, P., Behr, C., Avoli, M., 2013. Temporal lobe epileptiform activity following systemic administration of 4-aminopyridine in rats. *Epilepsia* 54, 596–604. doi:10.1111/epi.12041
- Nazer, F., Dickson, C.T., 2009. Slow oscillation state facilitates epileptiform events in the hippocampus. *J. Neurophysiol.* 102, 1880–1889. doi:10.1152/jn.90795.2008
- O'Neill, P.-K., Gordon, J.A., Sigurdsson, T., 2013. Theta oscillations in the medial prefrontal cortex are modulated by spatial working memory and synchronize with the hippocampus through its ventral subregion. *J. Neurosci. Off. J. Soc. Neurosci.* 33, 14211–14224. doi:10.1523/JNEUROSCI.2378-13.2013
- Patel, J., Fujisawa, S., Berényi, A., Royer, S., Buzsáki, G., 2012. Traveling theta waves along the entire septotemporal axis of the hippocampus. *Neuron* 75, 410–417. doi:10.1016/j.neuron.2012.07.015
- Paxinos, G., Watson, C., 2007. *The Rat Brain in Stereotaxic Coordinates: Compact, Sixth Edition* 6th Edition, Sixth Edition. ed.
- Paz, R., Bauer, E.P., Paré, D., 2008. Theta synchronizes the activity of medial prefrontal neurons during learning. *Learn. Mem. Cold Spring Harb.* N 15, 524–531. doi:10.1101/lm.932408
- Queiroz, C.M., Gorter, J.A., Lopes da Silva, F.H., Wadman, W.J., 2009. Dynamics of evoked local field potentials in the hippocampus of epileptic rats with

- spontaneous seizures. *J. Neurophysiol.* 101, 1588–1597.  
doi:10.1152/jn.90770.2008
- Reddy, D.S., Kuruba, R., 2013. Experimental models of status epilepticus and neuronal injury for evaluation of therapeutic interventions. *Int. J. Mol. Sci.* 14, 18284–18318. doi:10.3390/ijms140918284
- Scheffzük, C., Kukushka, V.I., Vyssotski, A.L., Draguhn, A., Tort, A.B.L., Brankač, J., 2011. Selective coupling between theta phase and neocortical fast gamma oscillations during REM-sleep in mice. *PloS One* 6, e28489.  
doi:10.1371/journal.pone.0028489
- Sedigh-Sarvestani, M., Thuku, G.I., Sunderam, S., Parkar, A., Weinstein, S.L., Schiff, S.J., Gluckman, B.J., 2014. Rapid eye movement sleep and hippocampal theta oscillations precede seizure onset in the tetanus toxin model of temporal lobe epilepsy. *J. Neurosci. Off. J. Soc. Neurosci.* 34, 1105–1114.  
doi:10.1523/JNEUROSCI.3103-13.2014
- Sirota, A., Montgomery, S., Fujisawa, S., Isomura, Y., Zugaro, M., Buzsáki, G., 2008. Entrainment of neocortical neurons and gamma oscillations by the hippocampal theta rhythm. *Neuron* 60, 683–697.  
doi:10.1016/j.neuron.2008.09.014
- Sitnikova, E., van Luijelaar, G., 2009. Electroencephalographic precursors of spike-wave discharges in a genetic rat model of absence epilepsy: Power spectrum and coherence EEG analyses. *Epilepsy Res.* 84, 159–171.  
doi:10.1016/j.eplepsyres.2009.01.016
- Sloviter, R.S., 2005. The neurobiology of temporal lobe epilepsy: too much information, not enough knowledge. *C. R. Biol.* 328, 143–153.
- Sloviter, R.S., Zappone, C.A., Bumanglag, A.V., Norwood, B.A., Kudrimoti, H., 2007. On the relevance of prolonged convulsive status epilepticus in animals to the etiology and neurobiology of human temporal lobe epilepsy. *Epilepsia* 48 Suppl 8, 6–10.
- Tang, F.R., Loke, W.K., 2010. Cyto-, axo- and dendro-architectonic changes of neurons in the limbic system in the mouse pilocarpine model of temporal lobe epilepsy. *Epilepsy Res.* 89, 43–51. doi:10.1016/j.eplepsyres.2009.10.015
- Tort, A.B.L., Komorowski, R., Eichenbaum, H., Kopell, N., 2010. Measuring phase-amplitude coupling between neuronal oscillations of different frequencies. *J. Neurophysiol.* 104, 1195–1210. doi:10.1152/jn.00106.2010
- Tort, A.B.L., Kramer, M.A., Thorn, C., Gibson, D.J., Kubota, Y., Graybiel, A.M., Kopell, N.J., 2008. Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. *Proc. Natl. Acad. Sci. U. S. A.* 105, 20517–20522.  
doi:10.1073/pnas.0810524105
- Zheng, C., Zhang, T., 2015. Synaptic plasticity-related neural oscillations on hippocampus-prefrontal cortex pathway in depression. *Neuroscience* 292, 170–180. doi:10.1016/j.neuroscience.2015.01.071

## Figure legends

**Figure 1: Experimental protocol of epilepsy model induced by intermittent long-term stimulation.** A: Brain regions where electrodes were implanted. Recording monopolar electrodes were implanted in the hippocampus DG, and monopolar

stimulation electrodes placed in the PP. Micro-screws previously soldered to silver wires were implanted over the cerebellum and the contralateral parietal cortex used as ground and stimulus reference, consecutively. **B**: Example of stimulation trains (b) followed by hippocampal afterdischarges (a) during PP stimulation. **C**: Stimulation protocol applied in freely-moving animals: 10-s trains of 20-Hz stimuli (200 monophasic of 1ms pulses) once each 50 seconds interval, for 8 hours, totalizing 480 stimuli trains (adapted from Sloviter et al., 2007). **D**: Spontaneous seizures LFP recording in mPFC and hippocampus. The epoch in red indicates the onset and ending of the seizure.

**Figure 2. Theta oscillations in the hippocampus decrease after seizures.** **A**. Raw LFP recording examples from the hippocampus and mPFC before and after an ictal event. **B**. Mean $\pm$ SEM (solid $\pm$ dashed lines) PSD of 120s LFP segments from hippocampus and mPFC before (pre, black lines) and after (post-ictal, red lines) seizures. Black dashed lines indicate the segment used in the insets. (Insets: 4-10 Hz PSD). **C**. Mean spectrograms showing 120s of hippocampus and mPFC recordings immediately before and after seizures (31 recorded seizures).

**Figure 3. Theta/Delta power increase prior to seizure onset in the hippocampus LFP.** **A**. Instantaneous theta amplitude calculated from the Hilbert transform of theta-filtered signals of hippocampus and mPFC 120s LFP segments. **B**. Theta/Delta power ratios of LFP segments in the 90-120s and 0-30s intervals before SRS. Significant increase prior to seizures is observed (\* $p < 0.05$ ) comparing hippocampus mean LFP segments (red line) of each interval. **C**. Example of a spectrogram from a 120s-segment of hippocampal LFP recording (left panel) and mean spectrogram of pre-ictal segments ( $n=31$  seizures – right panel).

**Figure 4. Theta coherence between hippocampus and mPFC increases pre-ictally.** **A**. Mean $\pm$ SEM (dashed lines) coherence of 30s LFP segments from hippocampus and mPFC before (pre, black lines) and after (post-ictal, red lines) seizures. **B**. Mean $\pm$ SEM (solid $\pm$ dashed lines) coherence of 30s recordings 120s (blue lines) and 30s (green lines) before seizures. **C**. Mean coherogram (coherence vs. time, 0-20 Hz) of pre and post-ictal LFP from hippocampus and mPFC. **D**. Blox plots of mean theta coherence after SRS in 31 analyzed seizures (left panel, \*  $p < 0.001$ ) and significant increase from 90-120s to 0-30s intervals, before seizures (right panel, \*  $p < 0.001$ ).

**Figure 5. Hippocampus/mPFC theta coherence decreases abruptly following seizure onset.** **A**. Example of 5-min (to show the emergence of theta activity) LFP recordings from mPFC and hippocampus recordings, ictal (highlighted in red) and 3 minutes post-ictal activity (showing post-ictal depression). **B**. Respective spectrogram from the hippocampus LFP and coherogram. White box highlights the drop in theta coherence with seizure onset. **C**. Mean theta coherence 30s before seizure and the decreasing during seizure (\*  $p=0.00005$ ). **D**. Mean theta coherence 30s before seizure and during inter-ictal theta (\*  $p=0.01$ ).

**Figure 6. Hippocampus/mPFC theta coherence increases independently of dorsal hippocampal theta power.** **A**. Pre-ictal and inter-ictal (LFP recording unrelated to seizures) spectrograms examples of hippocampal theta power (upper panels) and respective hippocampus/mPFC coherograms. **B**. Examples of correlations between

pre-ictal and inter-ictal hippocampal theta power and hippocampus/mPFC theta coherence (same traces as A). **C.** Bar graph showing mean correlation coefficient ( $r$ ) from pre-ictal and inter-ictal hippocampal theta power and hippocampus/mPFC theta coherence (\*  $p=0.003$ ).

**Figure 7. Hippocampus theta modulates mPFC and hippocampus gamma oscillations immediately prior to seizure onset.** **A.** Example of comodulation maps of hippocampal theta phase modulating hippocampal gamma oscillation amplitude 120s and 30s before seizure onset. **B.** Box plot showing mean theta/gamma modulation index for hippocampal LFP 120s and 30s before seizure onset (\*  $p=0.05$ ). **C.** Example of comodulation maps of hippocampal theta phase modulating mPFC gamma oscillation amplitude 120s and 30s before seizure onset. **D.** Box plot showing mean hippocampal theta/mPFC gamma modulation index, 120s and 30s before seizure onset (\*  $p=0.04$ ).

**Figure 8. The hippocampal mean theta directionally influences mPFC theta immediately prior to SRS onset.** **A.** Examples of theta band filtered LFP signals from the hippocampus and mPFC 120s and 30s before seizure onset. **B.** Examples of spectral Granger causality coefficients for the two cases shown in the top panels 120s and 30s in the pre-ictal segments. Confidence intervals (dashed lines) were produced using a non-parametric bootstrapping method (using the `bootstrap_tsdata_to_spwgcg` command from the multivariate Granger causality Matlab toolbox, Barnett and Seth, 2014) **C.** Mean $\pm$ SEM spectral Granger causality, when mPFC LFP Granger causes hippocampus LFP (black lines) and vice versa (red lines), 120s (left) and 30s (right) before seizure onset. **D.** Mean of maximum Granger theta power values 120s (left) and 30s (center) in the pre-ictal period, showing significant causality increase in hippocampus- mPFC direction, immediately before seizures (\*  $p=0.02$ ). In the 90-120s period before seizure, the difference was not observed in Granger causality. Significant increase in Granger causality was observed in hippocampus-mPFC direction, 30s before seizures in relation to the 120s pre-ictal period (right, \* $p=0.03$ ).

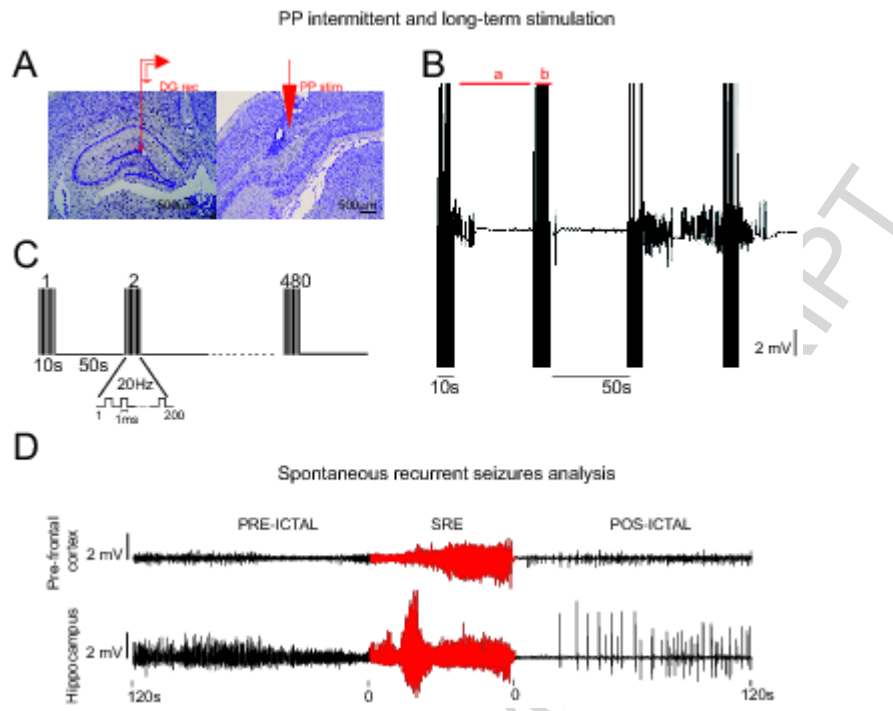


Figure 1



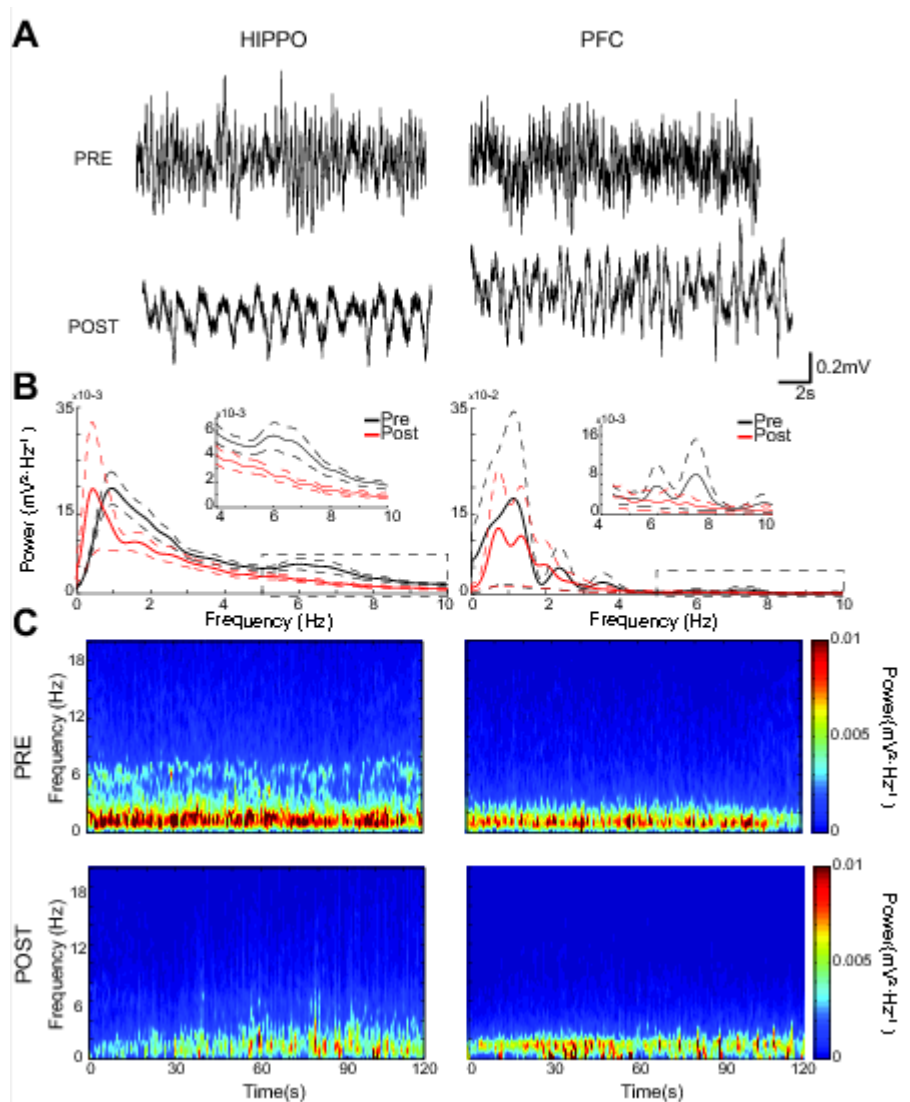


Figure 2

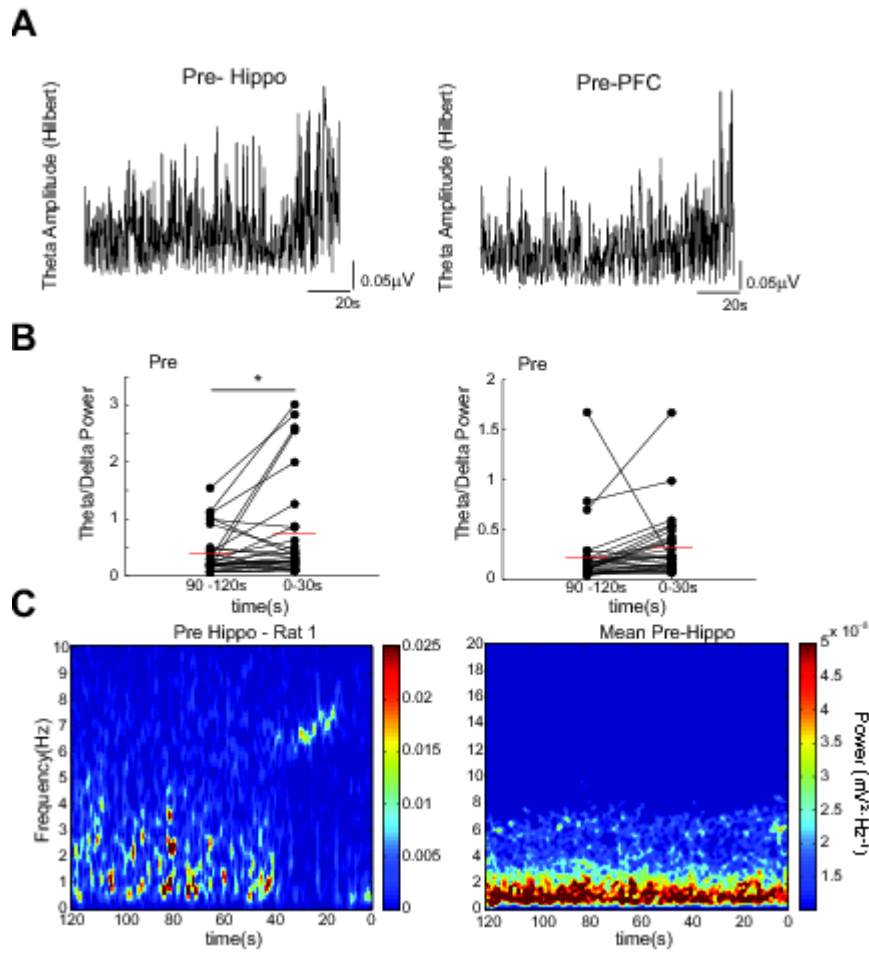


Figure 3

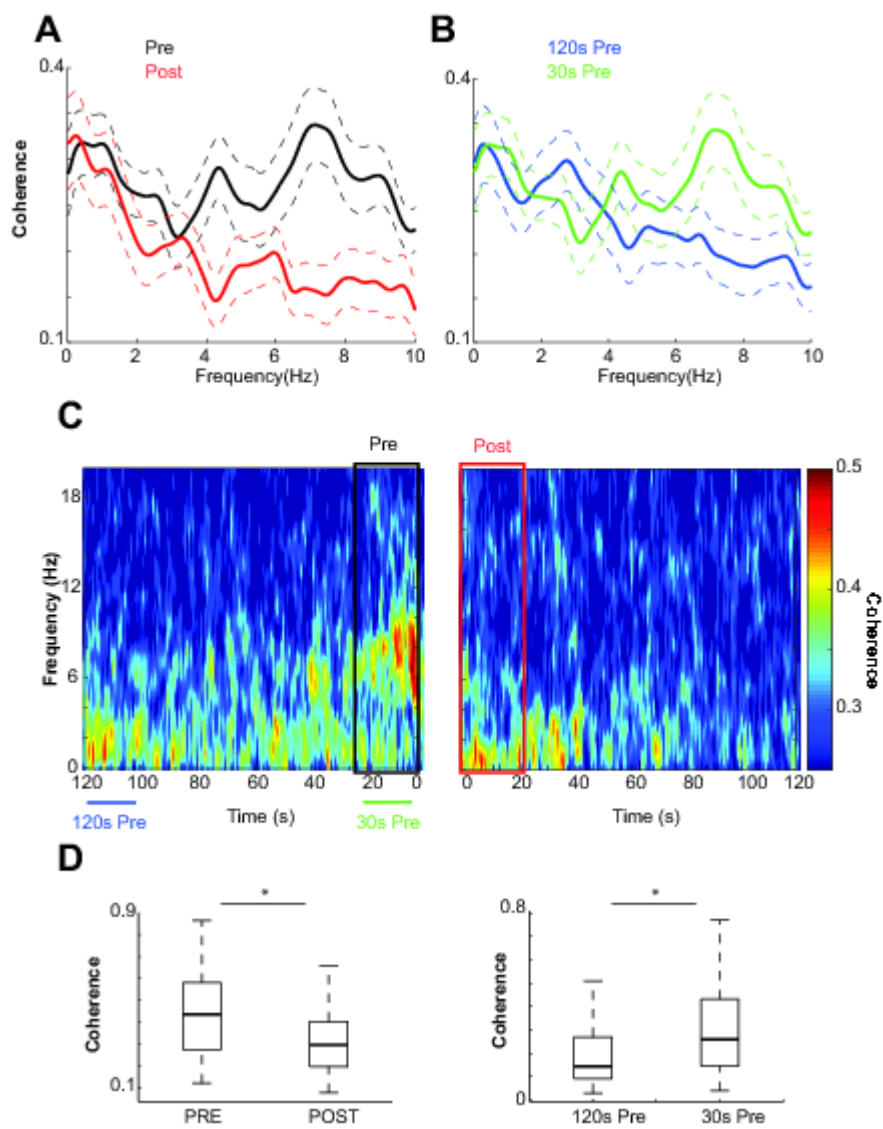


Figure 4

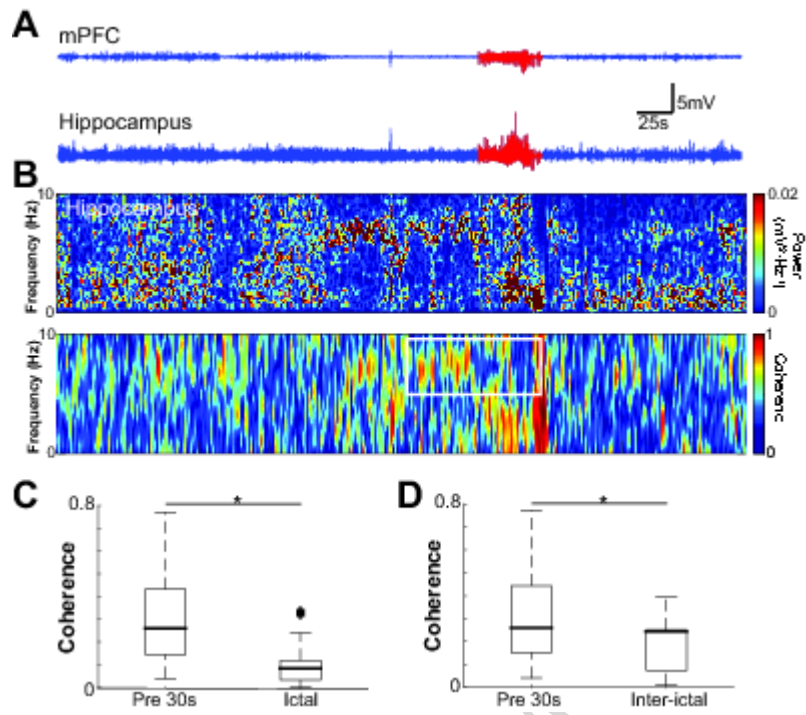


Figure 5

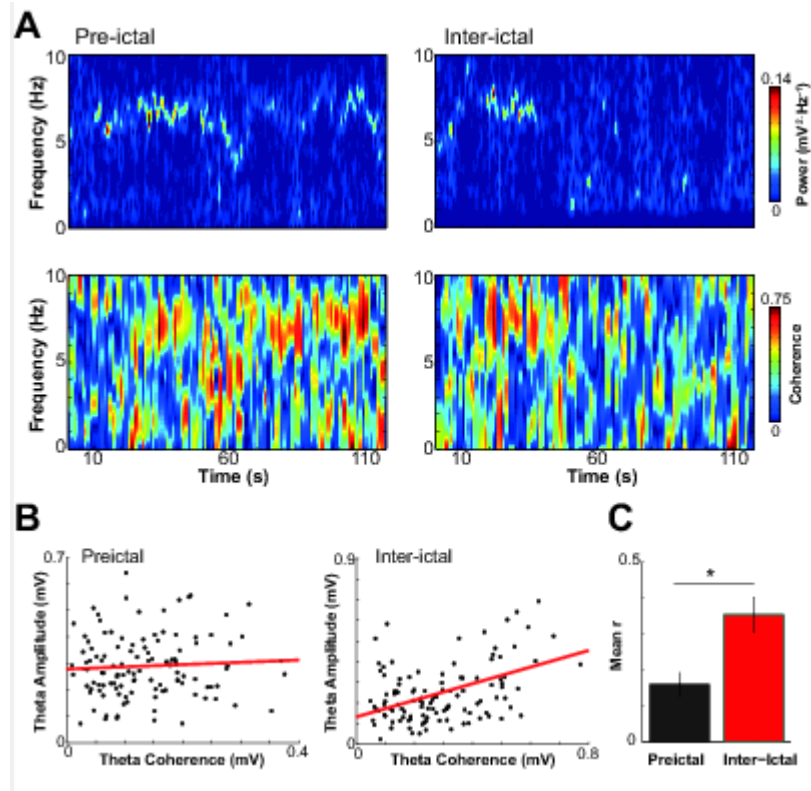


Figure 6

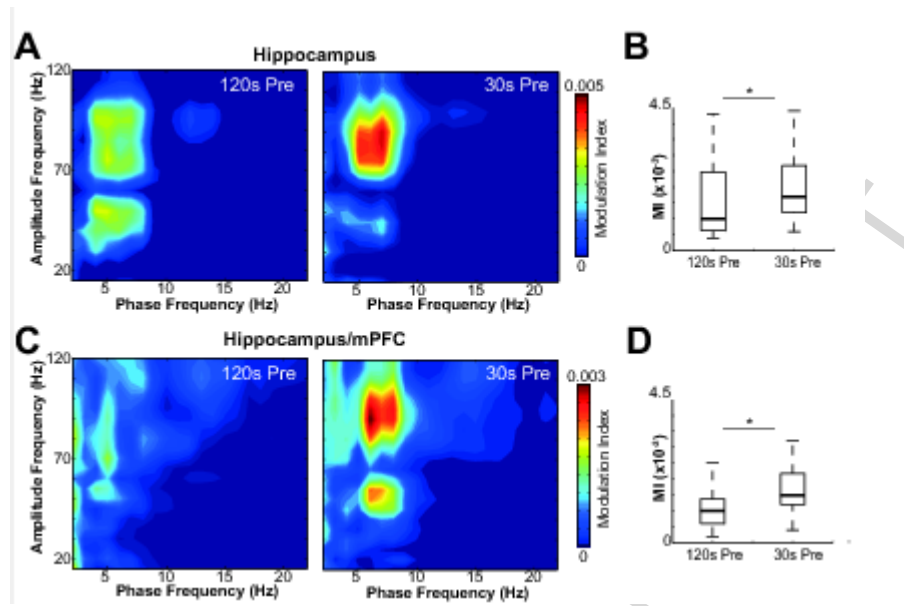


Figure 7

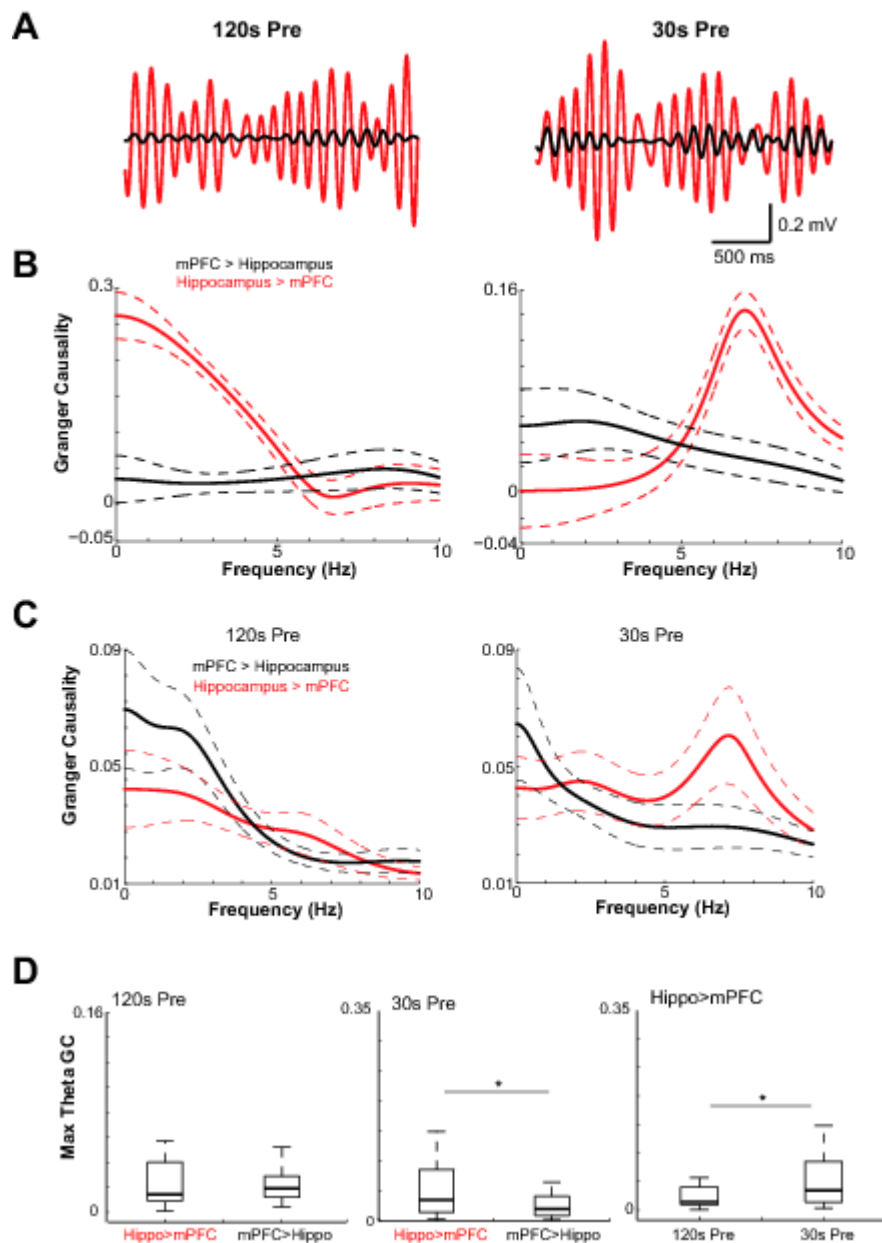


Figure 8

### Highlights

- Dorsal hippocampus and mPFC synchrony in theta increases immediately prior to seizure onset
- This increase in synchrony is accompanied by a stronger coupling between hippocampal theta and mPFC gamma oscillation
- The hippocampal mean theta directionally influences mPFC theta before seizure onset
- Theta coherence may be used in seizure prediction study and comprehension of spontaneous seizures generation network mechanisms

ACCEPTED MANUSCRIPT