The slow oscillation state facilitates epileptiform events in the hippocampus

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Abstract

In mesial temporal lobe (MTL) epilepsy, which typically involves the hippocampus (HPC), epileptiform events are enhanced during slow wave sleep (SWS). It remains unclear how and why the EEG states that constitute SWS might predispose the HPC to this type of pathological activity. Recently, our laboratory has described a novel state of deactivated hippocampal EEG activity that occurs during both SWS and urethane anesthesia: the slow oscillation (SO). This activity is characterized by a high amplitude ~1Hz signal, high synchrony within the hippocampus, and a dynamic coordination with neocortical SO. In order to assess how this activity state might influence epileptiform discharges, we studied the properties of stimulation evoked and spontaneous epileptiform events elicited in the HPC of urethane-anaesthetized rats. We compared those elicited during the SO to those occurring during the theta rhythm. The average duration but not the amplitude of evoked afterdischarges (ADs) was consistently larger during the SO. In addition, spontaneous epileptiform events were more frequent and of higher amplitude during the SO. Lastly, the bilateral propagation of both ADs and spontaneous events in the hippocampus was enhanced during the SO. These results imply that the threshold for the generation and propagation of epileptiform activity in the hippocampus is lowered during the SO and that this state may be a seed for the initiation, maintenance, and generalization of MTL epilepsy. Further examination of the pathophysiology of sleep-epilepsy interactions in the HPC will be of benefit for an understanding of the mechanisms, prognosis, and therapy for this form of epilepsy.
Introduction

Epilepsy is a neurological disorder in which patterns of hypersynchronized neuronal discharges (seizures) occur across large numbers of brain cells. These events are accompanied by gross disturbances of brain function and can be associated with violent and rhythmic motor behaviors (convulsions). The tendency for seizure-related activity is modulated by brain state, in most cases being more prominent during sleep (for reviews: Dinner 2002; Foldvary-Schaefer and Grigg-Damberger 2006; Kellaway 1985; Kotagal and Yardi 2008; Mendez and Radtke 2001). The relationship of sleep to epilepsy is unclear but one hypothesis suggests that certain patterns of physiological brain activity may predispose neuronal tissue to the hyperexcitability and/or the hypersynchrony involved in a seizure (Kellaway 1985; Steriade et al. 1994).

Sleep itself is composed of different stages, in humans, these can be broken up into stages 1 through 4 and paradoxical (or rapid-eye-movement - REM) sleep. Stages 1-4 are collectively regarded as non-REM and stages 3 - 4 are specifically referred to as slow-wave sleep (SWS). During REM sleep the neocortical EEG pattern tends to show small amplitude fast frequency components that are only locally synchronized (similar to those patterns observed during waking behavior; hence paradoxical) while during non-REM sleep the neocortical EEG is dominated by high amplitude slow frequency patterns which are more globally synchronized (Pace-Schott and Hobson 2002; Steriade and Amzica 1998). Interestingly, ictal and interictal epileptiform discharges tend to be more prevalent during the non-REM as opposed to the REM stage of sleep (Dinner 2002; Foldvary-Schaefer and Grigg-Damberger 2006; Kellaway 1985; Kotagal and Yardi 2008; Mendez and Radtke 2001). Thus, the high temporal and spatial synchronization of
activity commensurate with non-REM might be an important predisposing factor for 
epileptiform activities and seizures (Steriade and Amzica 2003). Obviously, knowledge 
of the functional relationship between physiological and pathological brain rhythms 
would allow for better medical interventions for seizure disorders.

An important form of epilepsy in this regard, is that which derives from medial 
temporal lobe structures. Alterations of consciousness and memory impairment are 
disturbing consequences of MTL epilepsy. Often pharmaco-resistant, MTL epilepsy 
tends to be the most frequently diagnosed in adults and requires expensive and invasive 
surgical interventions. The primary focus for MTL epilepsy is frequently within the 
hippocampal formation and hippocampal sclerosis is the most common anatomical 
pathology (Engel 2001). Animal models of MTL epilepsy often take advantage of the low 
hippocampal threshold for evoking seizure-like activity to create both chronic and acute 
forms of epileptic disorders (Morimoto et al. 2004).

Recently, our laboratory has described a novel state of collective hippocampal 
activity appearing during SWS and urethane anesthesia; the slow oscillation (SO) 
(Wolansky et al. 2006). This state is characterized by a high-amplitude rhythmic ~1Hz 
signal, which is highly synchronized within the HPC and can also be transiently 
synchronized with a similar pattern of slow oscillatory activity in the nCTX. Perhaps 
notable for the link between SWS and epilepsy, we also found that synaptic transmission 
in a majority of hippocampal pathways was facilitated during the SO (Schall et al. 2008).

Based on the known relationship of other forms of epilepsy to SWS we were interested in 
determining whether the properties of seizure-like discharges in the HPC were different 
during the SO as opposed to more activated rhythmic patterns like theta. To investigate
this relationship, we stimulated the hippocampus (HPC) of urethane-anaesthetized rats with kindling-like parameters during spontaneous rhythmic states characteristic of those appearing during REM and non-REM sleep (theta and SO, respectively). By measuring characteristics of the afterdischarges (ADs) evoked by this type of stimulation across states we were able to differentiate facilitative aspects of the SO state. Specifically, our results demonstrate that the SO state facilitates the generation, maintenance and propagation of epileptiform activities.
Materials and Methods

Data were obtained from 10 male Sprague Dawley rats weighing 190-274 (average ± SEM, 214.0 ± 10.0 g). All methods conformed to the guidelines established by the Canadian Council on Animal Care and the Society for Neuroscience and were approved by the Biosciences Animal Policy and Welfare Committee of the University of Alberta.

Anesthesia and surgery

Animals were initially induced with gaseous isoflurane at a minimum alveolar concentration (MAC) of 4 in an enclosed anesthetic chamber. After loss of righting reflexes, they were maintained on isoflurane (2.0-2.5 MAC) via a nose cone and implanted with a jugular catheter. Isoflurane was discontinued, and general anesthesia was achieved using slow intravenous administration of urethane (0.8 g/ml; final dosage, 1.29 ± 0.1 g/kg) via the jugular vein. Body temperature was maintained at 37°C using a servo-driven system connected to a heating pad and rectal probe (TR100, Fine Science Tools, Vancouver, Canada) for the remainder of the surgical and recording procedures. Level of anesthesia was assessed during the experiment by monitoring reflex withdrawal to a hindpaw pinch. If any visible withdrawal occurred, the animal was administered a supplemental dose (0.01 ml) of urethane until no longer responsive. Typically, once attaining surgical plane, and being placed in the stereotaxic apparatus, animals did not require further supplemental doses.

Stereotaxic placement of fixed recording electrodes was conducted for frontal neocortical and hippocampal sites as described previously (Wolansky et al. 2006). In brief, recording electrodes were constructed from Teflon-coated stainless steel wire (125
µm; A-M Systems). For bipolar recordings, we implanted bipolar electrodes assembled from twisting together two Teflon-insulated stainless steel wires (110 µm bare diameter, vertical tip separation of 0.5 - 1.0mm). These electrodes were aimed at superficial layers of frontal neocortex, (AP, +2.5; ML, ±1.2 mm; DV, -0.1 to -0.25 mm); and bilaterally at the level of hippocampal fissure of the dorsal HPC (AP, -3.3; ML, ± 2.2; DV, -2.8 to -3.3 mm). For stimulation electrodes we used two Teflon-coated stainless steel wire (200µm; A-M Systems) twisted together with a vertical separation of 0.5 - 1.0mm. The bipolar stimulating electrode targeted the hippocampal fissure of the dorsal HPC just posterior and lateral to the ipsilateral recording electrode (AP, -3.4; ML, ± 2.3; DV, -3.0 to -3.9 mm; HPC fissure, unilateral). After implantation, electrodes were fixed to the skull using jeweler’s screws and dental acrylic.

Multichannel field recordings were made using 16-contact silicon multiprobes arranged in a linear array with a contact separation of 100 µm (Neuronexus Ann Arbor, MI). Multiprobes were inserted vertically in the hippocampus using the same anterior-posterior and medial-lateral coordinates as single electrode hippocampal placements (AP: -3.3; ML: 2.2). The vertical (dorsal-ventral) positioning of the probe in the vertical plane was such that the span of contacts passed through stratum oriens (SOr) of CA1 through stratum granulosum (SGran) of the upper blade of the dentate gyrus (DG; tip at DV: 3.5 ± 0.2 mm).

**Recording procedures**

All single electrode field potential recordings were amplified at a gain of 1000 and filtered between 0.1 and 500 Hz using a differential AC amplifier (model 1700; A-M Systems). Signals from the multiprobe were amplified at a final gain of 1000 and wide-
band filtered between 0.7 and 1000 kHz via a 16-channel headstage (unity gain) and amplifier system (Plexon, Dallas, TX). All field signals were referenced to stereotaxic ground.

Stimulating procedures

The stimulating current was a monophasic cathodal pulse train with a pulse width of 1.0 ms, frequency of 100 Hz, and train duration of 1.0 s generated by using an isolated constant current pulse generator (model 2100; A-M Systems INC, Carlsborg WA, USA). This pattern of stimulation was derived from prior kindling experiments (Ozen et al. 2008). Stimulus intensity was established by determining the threshold for evoking ADs (range: 500μA to 5mA). The current intensity was gradually increased from 50 μA, stepwise by 50 μA, every 5 minutes to determine the threshold for eliciting focal epileptiform activity (after-discharge) in the HPC site. This just-threshold current intensity was used in subsequent experimental stimulations. Stimulation was conducted within each of the SO and theta states with a minimum repetition of 8 times per state. An adequate delay (on average 10.67 ± 1.36 minutes) was interposed between subsequent stimulation time points to allow ADs to subside and normal EEG patterns to re-emerge. In each case we waited until spontaneous and physiological EEG state changes occurred to ensure that EEG patterns were normal.

Data storage

All field signals were digitized with a Digidata 1322A analog-to-digital board connected to a Pentium computer running the AxoScope acquisition program (Molecular Devices, Union City, CA) using a sampling frequency of 1 KHz. All experiments were digitized on-line.
Perfusion and histology

After recording sessions, small lesions were made at the tips of recording and stimulation electrodes by passing 0.1-1 mA of DC for 5 s using an isolated constant current pulse generator (model 2100; A-M Systems). The multiprobe was moved slightly in both the AP and ML planes in order to better visualize the track. Rats were perfused transcardially, initially with physiological saline and then with 4% paraformaldehyde in saline. Brains were extracted and stored overnight in 30% sucrose in 4% paraformaldehyde. The tissue was frozen with compressed CO₂ and sliced at 48 µm with a rotary microtome (1320 Microtome; Leica, Vienna, Austria). Slices were then mounted on gel-coated slides, allowed to dry for a minimum of 24 h, subsequently stained using thionin, and coverslipped. Microscopic inspection of stained slices was used to verify recording and stimulating loci. Digital photomicrographs (Canon Powershot S45; Canon, Tokyo, Japan) were taken on a Leica DM LB2 microscope, imported using Canon Remote Capture 2.7 software and processed with Corel PhotoPaint (Corel, Ottawa, Ontario, Canada).

Data processing and analysis

Raw EEG was visualized with AxoScope (Molecular Devices). Segments of raw EEG, which contained the pre-stimulation period, after discharge period and recovery period, were selected for analysis. Matlab version 5.3 (MathWorks, Natick, MA) was used to analyze signals in both the time and frequency domains. The results were visualized using Origin (Microcal Software, Northampton, MA). Spectral analysis on the pre-stimulation period was performed to confirm the EEG state as described in (Wolansky et al. 2006). In brief, spectra were computed using Welch’s periodogram
method. The calculation was performed on sequential Hanning windowed samples of 6 s length with 2 s overlap. Spectral were inspected for characteristics of the activated (theta) or deactivated (SO) state in terms of the concentration of peak logarithmic power in the 3-5 Hz versus 0.5-1.5 Hz bandwidths, respectively (Figure 1D: see also (Wolansky et al. 2006)). Spectrograms were computed for signals longer than 30s using a windowing method (Clement et al. 2008; Wolansky et al. 2006). In brief, windows of 24 s in duration were moved across the data segment in increments of 6 s and power spectra were computed for each window as described above. In most cases, the temporal fluctuations of the log-transformed power values within the 0.5-1.5 Hz bandwidth of spectrograms were sufficient to characterize state (Clement et al. 2008). In some cases, the ratio of the log-transformed power values in the SO versus the theta bandwidths was used to determine state changes.

AD duration was measured from the onset of the stimulation to the last spike of the AD event. AD spikes were detected both visually and triggered automatically based on their amplitude. Trigger amplitudes were based on values that were greater than the ninety-fifth percentile of the amplitude distribution of the EEG signal itself. The average amplitude of ADs was calculated in two ways: The first involved computing the root mean square (RMS) (i.e. the standard deviation) of every six-second window of the primary AD and calculating the average across the entire duration. The second involved integrating the power spectral density between 0 and 500Hz of the first eighteen seconds of primary ADs.

The inter-site propagation of ADs was analyzed by computing and comparing the spectral coherence of both physiological and AD activity across different signals. We
concentrated our analyses to the 10-100 Hz bandwidth since this bandwidth shows low coherence during physiological states and the greatest increases during epileptiform activity. In order to confirm that our results were not artificially inflated due to a common reference (in this case sterotaxic ground) (Biggins et al. 1991) we performed recordings using simultaneous monopolar and bipolar recordings and repeated the coherence analysis. We found that the average inter-hippocampal coherence (between 10 to 100 Hz) was not significantly different between monopolar (theta: 0.1 ± 0.01; SO: 0.11 ± 0.04) and bipolar (theta: 0.06 ± 0.02; SO: 0.06 ± 0.01) recordings across states (pairwise one-tailed t test: p > 0.05; N =4; data not shown).

Following repeated ADs, spontaneous epileptiform events could occur. Spontaneous epileptiform events were detected visually and using the same triggering methods as described above. Across states (as defined using the spectral methods above) the frequency of events was expressed as the ratio of events per elapsed time. These were averaged and compared across states. Likewise, the peak-to-peak amplitude of each event was measured directly and averaged for each state. Since spontaneous events were shorter in duration than ADs, the propagation of these events was estimated by computing the crosscorrelogram of high-pass filtered (30 Hz) events. The correlation values at the first and second positive peaks closest to the origin (zero lag) were averaged across different instances within states.

 Probe mapping methods

We used the multiprobe to record spontaneous SO and theta activity as well as evoked and spontaneous epileptiform activities from the dorsal HPC. This method allowed us to construct a laminar voltage profile and to compute the current source
density (CSD) profile of spontaneous and evoked EEG signals of the hippocampus. A power and cross spectral phase profile for each channel of the probe (16 total) was calculated across SO and theta states (Wolansky et al. 2006). Power spectra were computed as described above for monopolar recordings. Cross phase spectra were computed by comparing each channel from the probe against the fixed contralateral hippocampal signal. By extracting values at 1.0 Hz for SO and at (on average) 3.5 ± 0.2 Hz for theta, both the power and cross phase could be plotted as a function of depth. The position of each contact could be identified based on the topography of these profiles (Wolansky et al. 2006) in combination with the histological track.

Current source density analysis

The spatial and temporal distribution of current sources and sinks underlying particular voltage profiles were computed for spontaneous field potential profiles recorded using the linear multiprobe. The underlying assumptions for CSD followed those of prior work (Freeman and Nicholson 1975; Ketchum and Haberly 1993; Nicholson and Freeman 1975; Rodriguez and Haberly 1989). CSD was computed by estimating the second spatial derivative of voltage traces derived from the multiprobe. This estimate was calculated using a three-point difference (differentiation grid size of 300 µm) on the voltage values across spatially adjacent traces and expressed as mV/mm²:

$$CSD = \frac{f(p_{i-1}) - 2f(p_i) + f(p_{i+1})}{d^2},$$

Where $f(p_i)$ is the field signal from probe channel $i$ ($i = 2, 3, ..., 15$), and $d$ is the distance between adjacent channels (0.1 mm). For traces at each end of the probe (e.g., channels 1 and 16), the differentiation grid was based only on the immediately adjacent channel (e.g., channels 2 and 15, respectively). We confirmed that this procedure yielded similar,
if not identical, CSD results as the 3-point differentiation method by successively eliminating probe end channels and then re-computing and comparing results.

Statistics

To compare values across states, we used pairwise one-tailed t tests (p < 0.01). Averages are reported as arithmetic means ± the standard error of the mean (SEM). The number of cases for averages are represented in uppercase (N) when referring to number of animals whereas lowercase (n) refers to number of instances per animal.

Drugs and chemicals

Thionin, and urethane were all purchased from Sigma (St. Louis, MO). Isoflurane was purchased from Bimeda MTC Animal Health (Cambridge, Ontario, Canada), paraformaldehyde from Fisher Scientific (Toronto, Ontario, Canada).
Results

Histological findings

We confirmed the location of all single electrode and multiprobe locations. All single electrode recording positions were found in the stratum radiatum (SRad) layer of the CA1, stratum lacunosum-moleculare (SLM) just above the hippocampal fissure, and less often in stratum moleculare (SMol) of the dentate gyrus. These positions are summarized in Figure 1A. The termination of probe tracts was typically in the hilar region of the DG just ventral to the granule cell layer and the tracks themselves passed completely through all the layers of CA1 as shown in Figure 1B. The position of individual contact sites was estimated from the orientation of the histological tract in combination with comparisons to the distribution of spontaneous (theta and SO) profile measures (Wolansky et al. 2006). We also confirmed the location of all stimulation sites, which were found mainly in CA1 and the DG. One site was at the level of curvature of CA3 (summary diagram of placements are shown in Figure 1C).

Spontaneous alternations of state-dependent activity in the hippocampus

As previously reported (Clement et al. 2008; Wolansky et al. 2006), the electrographic activity of the hippocampus under urethane anesthesia showed alternating cycles of activated and deactivated states (Figure 1D). The activated state was characterized by a moderately high amplitude (1.3 ± 0.13 mV peak to peak; N =7) theta rhythm (bandwidth of 3.3-3.7 Hz) whereas the deactivated state was characterized by a still higher amplitude (2.37 ± 0.11 mV peak to peak; N =7) SO at a lower frequency bandwidth centered around 1Hz (range of 0.8-1.0Hz). The neocortical recording demonstrated concomitant state alternations, which were characterized by prominent low
State-dependent alteration of evoked epileptiform activity (afterdischarges)

Because of the intensity of the current threshold for eliciting ADs across states and the fact that stimulation could only be conducted at most once during the spontaneous occurrence of any particular state, we were not able to systematically distinguish the threshold levels for evoking ADs by systematically varying current intensity. Thus, we used suprathreshold kindling-like stimulation parameters in both of these states and compared the resulting AD durations.

A characteristic AD consisted of a preliminary phase in which there was a multiphasic polarity shift that consistently began as a negative going potential and eventually returned to the zero line. This presumably resulted from a quickly developing but slowly decaying near-DC potential and lasted on average 7.62 ± 0.73 s (Figure 1D). Following this, there was the appearance of a train of bursts composed of spikes having an amplitude range of 15.1 ± 1.0 mV peak to peak and an average duration of 41.9 ± 3.35 s. Intraburst frequencies were 39.2 ± 10.2 Hz and interburst frequencies were 2.56 ± 0.1 Hz (N = 4). The bursts were more frequent in the beginning of AD train and declined towards the end, however the shape of individual spikes appeared similar across the train (Figure 1E). Following the train, a brief period (14.6 ± 5.4 s) of depressed (0.72 ± 0.06 mV peak to peak) electrographic activity occurred. Subsequent to the electrographic depression the field record was frequently dominated by low frequency band (~1 Hz) EEG activity of similar amplitude to the SO. The spectral analytical components of this signal were similar to the normal SO (average peak frequency 0.92 ± 0.8 Hz).

Occasionally, this period also demonstrated a secondary set of discharges characterized
by similar spikes as observed during the primary AD with an amplitude range of 2.5 ± 0.83 mV (peak to peak) and an average frequency of 17.4 ± 1.2 Hz. Following a variable period (197.5 ± 102.5 s), the electrographic activity returned to a typical cyclical alternation of physiological state dependent activity as described above. Although the neocortical recording showed an initial polarity shift followed by a limited set of primary spike discharges with lower amplitudes than those shown at hippocampal sites, the secondary discharges were not apparent.

Across states, we found some similarities but also some differences in the measures we obtained for evoked ADs. When the total duration of the ADs (including slow-multiphasic, primary and secondary discharges) were considered, there was a significant difference between states where the durations were longer in SO as compared to theta (SO: 80.7 ± 4.3 s; Theta: 53.2 ± 5.8 s; pairwise one-tailed t test: p < 0.01; N =7; Figure 2A). This was undoubtedly related to the fact that the secondary discharges were significantly more prevalent when evoked during the SO (75.0 ± 0.05% of cases) as opposed to theta (22.9 ± 0.2% of cases; pairwise one-tailed t test: p < 0.01; N =7). When the duration of the slow-multiphasic and the first primary train of discharges were compared across states, these were not significantly different (SO: 44.0 ± 5.46 s versus theta: 48.5 ± 4.74 s; pairwise one-tailed t test: p < 0.01; N =7).

Amplitude differences for ADs across states were evaluated by two measures; root mean square (RMS) derivations of raw AD traces and the integration (summation) of power values (0-500Hz) of AD spectra. The average RMS for ADs across experiments during SO was 1.15 ± 0.24 mV while during theta it was 1.0 ± 0.19 mV (Figure 2B). The average integral of spectral power for ADs across experiments during SO was 1137.5 ±
119.2 mV² while during theta it was 1428.8 ± 73.6 mV²; Figure 2C). Neither of these measures was significantly different across states (pairwise one-tailed t test: with p > 0.01; N = 6).

**Primary and secondary afterdischarges were generated within the hippocampus**

To confirm that ADs were generated within hippocampal circuitry, we performed simultaneous laminar recordings using the linear multiprobe positioned vertically through the hippocampus, straddling the CA1 and DG regions (Figure 3A; B). ADs were elicited using the same kindling-like stimulation as described before delivered to the ipsilateral HPC.

Similar to previous findings in other laboratories (Bragin et al. 1997; Wadman et al. 1992), primary ADs were distributed across all layers of CA1 and DG and showed systematic changes in amplitude with depth (Figure 3A). Indeed, examination of expanded elements of the AD (Figure 3B) showed a reversal point for slow components of ADs at stratum pyramidale (SPyr; 2.5 mm depth in Figure 3B). The fast components showed multiple reversal points: SPyr; SRad (2.8 mm depth in Figure 3B); and SMol in the DG (3.1 mm depth in Figure 3B). As well, CSD analysis of traces (Figure 3B) demonstrated multiple regions of sink-source alternations across the layers of CA1 and DG. The strongest sinks occurred in SRad (2.7 mm in Figure 3B) and in the hilar region of the DG just below and including SGran (3.3-3.4 mm depth in Figure 3B). However, there were other prominent sink zones in SOr (2.4 mm depth in Figure 3B) and SMol of the DG (3.2 mm depth in Figure 3B). Furthermore, there was a long-lasting sink apparent in SMol just below the HPC fissure (3.1 mm depth in Figure 3B), which occurred following the burst events. The locations and dynamics of CSDs for primary ADs were
well conserved both within and across animals.

Secondary ADs were also distributed across all layers of CA1 and DG, and also showed systematic changes in amplitude with depth (Figure 3C). Examination of expanded elements showed dual reversal points for the slow (wider) components of these ADs at two levels in SRad; one just below SPyr and another deeper (2.6 and 2.9 mm depth in Figure 3D, respectively). Faster components reversed at the level of SPyr as well as mid SRad (2.5 and 2.7 mm depth in Figure 3D, respectively). CSD analysis of traces (Figure 3D) demonstrated multiple regions of sink-source alternation across the laminae of CA1 and the DG. The strongest sinks occurred across the expanse of SRad and in some cases may have involved SLM (2.6-2.9 mm depth in Figure 3D). Weaker sinks were also present at the level of SPyr, SGran and in SMol. Based on these results we were confident that both primary and secondary ADs represented epileptiform events generated within the hippocampal network. The locations and dynamics of CSDs for secondary ADs were well conserved both within and across animals.

We were also able to characterize the slow activity expressed just following the termination of ADs using laminar profile analysis of both the field potentials and current source density traces. Interestingly, these events had a very different CSD spatial profile than physiological SO, which included prominent sinks at the level of SPyr (not shown). This suggests that they reflect a post-ictal as opposed to a physiological state of activity.

Propagation of ADs was facilitated during the SO

Given the finding of extended AD duration during the hippocampal SO, we also examined the intra-areal and extra-areal coordination of ADs across states. To perform these experiments we implanted a pair of electrodes at homotopic points bilaterally in the
hippocampi. As well, we included a cortical electrode implanted in the frontal region of cortex ipsilateral to stimulation. We evoked ADs in one HPC using kindling-like stimulation and assessed the coordination of the fast components (39.2 ± 10.2 Hz) of AD activity using coherence analysis. Although ADs consisted of both slow and fast components, in order to eliminate the influence of highly coherent physiological activity at lower frequencies, we concentrated on only the fast components that were unique to the epileptiform activity (Figure 4). Since we evoked ADs using unilateral hippocampal stimulation, we presumed that AD activity recorded at neocortical and contralateral hippocampal sites was due to propagation to and (potentially) engagement of AD activity at these sites.

Interhippocampal coherence values of the fast components of ADs, averaged across a frequency range from 10-100 Hz, were significantly higher for events elicited during SO as opposed to theta (SO: 0.44 ± 0.09; theta: 0.21 ± 0.03; pairwise one-tailed t test p < 0.01; Figure 4D). This was despite the fact that coherence values in the same range were not different across physiological states before ADs were elicited (SO: 0.14 ± 0.02; theta: 0.1 ± 0.01; pairwise one-tailed t test p > 0.01). Ipsilateral hippocampo-cortical coherence values of ADs, averaged across the same frequency range, however, were not significantly different across SO and theta (SO: 0.17 ± 0.04; theta: 0.22 ± 0.06; pairwise one-tailed t test p > 0.01).

**Spontaneous epileptiform events and state**

Following a series of AD-evoking stimulations, spontaneous epileptiform events could be observed at hippocampal recording sites (Figure 5). These events were composed of a train of field spikes having an average interspike interval of 4.78 ± 0.2 ms.
and spike amplitude range of 1.67 ± 0.2 mV. On average, individual events lasted 4.92 ± 0.13 s (Figure 5A). Typically, these events occurred in a series, which could last from a minimum of 8 to a maximum of 40 minutes. At the beginning of one of these epochs, each event was separated by 10.01 ± 0.1 s and this frequency steadily decreased until the events finally ceased.

These spontaneous events also gave us the opportunity to elaborate the association between states and epileptiform activity. Unfortunately, the number of epochs within (average 1.83 ± 0.048 per animal) and even across animals (total N =6) was too low to conduct a meaningful analysis of which state they were initiated in. However, since the epochs were long enough, we were able to calculate their average frequency by enumerating the number of individual events that occurred in either state per unit time during the entire epoch. Across animals the average occurrence was 0.75 ± 0.046 events per second during SO and 0.23 ± 0.068 events per second during theta. These values were significantly different (pairwise one-tailed t test with p < 0.01). Not only were the occurrences of spontaneous events higher in SO, but they also appeared to be larger in amplitude. By measuring the amplitude of these events at the site ipsilateral to stimulation these events were found to be 1.55 ± 0.15 times larger in SO as compared to theta (average amplitude during SO: 5.90 ± 0.24 mV vs 3.87 ± 0.55 mV during theta; Figure 5B;C).

Propagation of spontaneous epileptiform events was facilitated during the SO

Next, we assessed whether spontaneous epileptiform events showed differential properties in terms of contralateral coordination between hippocampi as a function of state. Again, we presumed that spontaneous events derived stemmed from a focus in the
hemisphere ipsilateral to stimulation and that it propagated contralaterally. To assess this presumed propagation we evaluated the cross correlation of high pass filtered events at ipsilateral and contralateral sites in the HPC. Since individual events were short, we could not use our averaged coherence analysis which required a larger time sample in order to produce meaningful results.

Cross correlation analysis of high pass filtered data showed rhythmic peaks occurring at a period of 4ms, which corresponded to the frequency of the fast spikelets. Interestingly, in most of our experiments the peak value of the cross correlation function was not at the instantaneous (0 ms lag) position but at the single cycle time shifted position (4ms lag), suggesting a systematic delay between the signals recorded across the two hippocampi. This time course is consistent with a monosynaptic feedforward synaptic connection and thus presumably reflects the interhemispheric propagation of individual spikelets and/or alternating reverberation between hippocampi. The ratio of the peak cross correlation values for theta and SO was computed for both states. The average ratio of values between SO and theta states at zero lag was calculated to be 1.46 ± 0.31 (SO > theta). Furthermore, the average ratio of values between SO and theta states at the 4ms lag was calculated to be 1.42 ± 0.28 (SO > theta; Figure 5D). The relative increase in correlation values during the SO demonstrates that the interhemispheric coordination (and presumed propagation) of spontaneous epileptiform events was enhanced during the SO.

*Spontaneous epileptiform events were generated within the HPC*

In order to confirm that the spontaneous epileptiform events were generated within the HPC we used the linear multiprobe as described previously. As with evoked
ADs, the potentials associated with these spontaneous events were distributed across the layers of both CA1 and DG with systematic amplitude changes associated with depth (Figure 6A;C). Expanded events showed a reversal point for the slow (wider) components of individual events in the proximal aspect of SRad (2.6 mm depth in Figure 6B;D) and for fast components at a more distal level of SRad (2.8 mm depth in Figure 6B;D) during both SO and theta states. CSD analysis of spontaneous events showed an initial and prominent sink in proximal SRad (2.7-2.6 mm depth in Figure 6B;D) with another later but still prominent sink region across SPyr and SOr (2.5-2.4 mm depth in Figure 6B;D). These regions could also show rhythmic (32.13 ± 3.72 Hz) sink source alternations following the initiation of individual events. The hilar region of the DG (3.5-3.6 mm depth in Figure 6B;D) also showed rhythmic (96.73 ± 1.02 Hz) sink source alternations throughout the episode. This pattern appeared similar across states, although the current flow was less during theta samples (see Figure 6B and D).

Discussion

Collectively, our results showed that the SO state in the HPC allowed for an enhanced maintenance and propagation of locally generated epileptiform events. Although we did not directly test whether the threshold for the initiation of these epileptiform events was lowered during the SO, our results suggest that this may be the case.

It is not clear why either the initiation or the maintenance of seizure-like activity would be facilitated by the hippocampal SO. It is certainly the fact, however, that MTL epileptics show an increased propensity to generate epileptic activity during slow wave
stages of sleep (Mayanagi 1977; Rossi et al. 1984; Sammaritano et al. 1991). Indeed, in neocortical regions, spike and wave and Lennox–Gastaut type discharges preferentially evolve from slow sleep oscillations (Steriade and Amzica 2003). It may be that the SO state simply enhances excitability which could allow for a biasing of hippocampal circuits towards hyperexcitability. In support of this conjecture, we have recently shown that synaptic excitability is enhanced during the SO in a majority of intrahippocampal pathways (Schall et al. 2008).

It may also be that the considerable physiological synchronization present during the SO state within the HPC (Wolansky et al. 2006) could allow for the facilitated development of pathological hypersynchrony. That is, the synchronized shifting of membrane potentials from down to up states and vice versa across a wide segment of the hippocampal population during the SO might predispose the circuitry to synchronized paroxysms. Certainly, it would be reasonable to presume that the propagation of epileptiform activity would be enhanced by existing synchrony. Future research directed at elucidating and dissociating these two potential mechanisms (ie. hyperexcitability versus hypersynchrony) would certainly lead towards effective strategies to control MTL seizures.

Phase effects

We have also recently shown that excitability throughout the hippocampal circuit is also modulated by SO phase (Schall et al. 2008). It would be of substantial interest, therefore, to understand if spontaneous epileptiform activities are more probable at any particular phase of the SO cycle as well. Since the stimulation technique that we used to elicit epileptiform events had a duration that was equivalent to one cycle of the SO we
were not able to answer this question directly in this study. Furthermore, the spontaneous epileptiform events that we assessed in our study were also too lengthy in duration and too well distributed across the hippocampi in order to assess any phase effects with ongoing SO. This latter point was critical since the amplitude of epileptiform events made it difficult to obtain accurate measurements of the phase of the ongoing slow field activity. Regardless, since it has been shown that it is the deeper stages of slow-wave sleep that tend to show more frequent interictal epileptiform activities (Sammaritano et al. 1991), it would be of substantial interest to confirm if short-duration events occur at a preferred phase of the ongoing SO. This would suggest that phase modulation of excitability or synchrony in the hippocampal network during the SO plays an important role in the generation of epileptiform events. Using a model system to mimic these short-duration events during spontaneous hippocampal SO would be most advantageous.

Clinical relevance

Our results support the importance of conducting sleep EEG for the diagnosis of MTL epilepsy. Since many non-generalized complex partial seizures occur more frequently during sleep (Dinner 2002), it effectively means that they can remain undetected by virtue of their lack of overt motoric symptoms, except through the use of electrographic methods. Our results suggest that it is the deepest stages of slow-wave sleep, which most prominently encourage hippocampal epileptiform events. Thus, the recordings with the greatest degree of benefit would be those that include at least a full sleep cycle. In addition, our results are in agreement with the use of sleep EEG for localization purposes (Buechler et al. 2008). REM sleep has been shown to have the highest value in this respect since it appears to restrict the propagation of interictal
activities (Sammaritano et al. 1991). Similarly in our study, we found that spontaneous
events tended to be less abundant and more restricted in terms of their propagation during
the theta state. Both of these facts emphasize the importance and relevance of
polysomnographic EEG studies in the diagnosis and localization of MTL epileptiform
events.

The interaction of sleep and epilepsy appears to be bidirectional in that the
architecture of sleep itself appears to be disrupted in epileptics (Bazil 2008). Specifically,
with early night seizures, the REM sleep proportion decreases almost to half of its
baseline measure (Bazil et al. 2000). Overall, partial epileptics (including those with
MTL pathologies) have twice the incidence of sleep disturbances than neurological
controls, which may indicate that sleep impairment could be in part due to the nature of
the disease (de Weerd et al. 2004). Altogether these findings suggest that sleep quality in
epileptics is impaired and this is most troubling when considering that sleep deprivation
itself has been shown to be pro-epileptic (Rodin et al. 1962). Regardless of the causal
links between sleep and seizure propensity, sleep or sleep-cycle disruption via epileptic
conditions may well increase the propensity of future MTL seizure attacks in an ongoing
repetitive sequence. This explains the rational for sleep hygiene enhancement in epileptic
patients, which results in both lifestyle improvement and disease control (Bazil 2008).

Lastly, the link between cognitive impairment and epilepsy may be partially due
to or exacerbated by sleep disruption. Impaired cognition has commonly been attributed
to altered physiological functioning of the epileptic brain or a side effect of anti-epileptic
treatments or both (Shulman and Barr 2002). However, it may also be due to altered sleep
patterns themselves (Motamedi and Meador 2003). Recently, the SO state has been
pinpointed as an important neural activity that gives rise to sleep-dependent consolidation of declarative (i.e., hippocampal dependent) memories (Bodizs et al. 2002; Marshall et al. 2006; Molle et al. 2009; Molle et al. 2004; Rasch et al. 2007). Our evidence that epileptic events are more prevalent during HPC SO patterns may indicate that the normal physiological synchronization presumed to underlie consolidatory processes during sleep during this stage is pathologically disrupted. An additional facet related to this would be any effect of anti-epileptic medication itself on sleep architecture. This would also be expected to interfere with normal physiology during sleep and might explain the net impairment of memory function in even early stages of MTL epilepsy.

Conclusions

Having demonstrated that the maintenance and the propagation (and quite possibly the threshold) for evoked and spontaneous epileptiform events is facilitated in the HPC during the SO, we suggest that this form of synchronized rhythm which appears normally during deep stages of slow-wave sleep, could promote electrographic seizures and epilepsy itself. The association of MTL seizures to HPC SO patterns has both diagnostic and therapeutic facets, which should be considered clinically. The disruption of normal EEG patterns and stages during sleep in epileptics could also be a fundamental causal element of cognitive and mnemonic impairment in these patients.

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References


Figure Captions

Figure 1: Histological sites for recording and stimulation and differential effects of oscillatory states in the hippocampus on afterdischarges (ADs). A. Single electrode recording sites across experiments. All sites were located near the hippocampal fissure. B. Multiprobe electrode tracks across experiments. All tracks passed through the CA1 pyramidal cell layer and the DG granule cell layer. C. The stimulation sites across experiments. All sites were in the hippocampus but were distributed across CA1, CA3 and the DG. D. Comparison of the raw, spectral and autocorrelational aspects of spontaneous theta versus SO in urethane anesthetized rats. Theta activity (top traces) demonstrates ~3 Hz rhythmicity in the raw signals (left panel), spectral plot (middle panel) and autocorrelogram (right panel). SO (bottom traces) demonstrates ~1 Hz rhythmicity in the raw signals (left panel), spectral plot (middle panel) and autocorrelogram (right panel). E. Comparison of the evoked ADs during theta (top) and SO (bottom). Insets demonstrate the similarity of rhythmic discharges during the AD in both. The duration of the primary AD during SO is slightly longer and there is also the occurrence of a secondary AD (see expanded inset below). The normative probability distributions of field potential amplitudes for both states are shown at the right of both traces.

Figure 2: Comparison of AD durations and amplitudes across states. The left panels show results from individual ADs evoked in the experiment shown in Figure 1E along with the overall averages as a function of state. The middle panel shows the matched averages across state for each experiment and the rightmost panel shows the overall averages (both normalized to theta) across states. A. The durations of ADs evoked
during the SO were significantly longer than the ADs during theta. B. The RMS values of ADs during SO were not significantly different to those evoked during theta. C. The integrated power values of ADs during SO were not significantly different to those evoked during theta.

Figure 3: Multiprobe profiles and CSDs of primary and secondary ADs. A. Multiprobe profile of a primary AD evoked during the SO state. B. Expanded traces of the primary AD show a reversal point for the slower components of events at SPyr and multiple reversal points for the fastest components at SPyr, SRad and SMol. CSD profiles show that the strongest sinks occur in SRad and in the hilar region of the DG just below and including SGran. C. Multiprobe profile of secondary ADs evoked during SO state. D. Expanded traces of secondary ADs show reversal points for the slow components at SRad and for the faster components at the level of SPyr and SRad. CSD profiles show multiple regions of sink-source alternation across the laminae of CA1 and the DG. The strongest sinks occur across the expanse of SRad and in some cases SLM.

Figure 4: Inter-hippocampal coherence as a measure of AD coordination (and implied interhemispheric propagation) across states. A. ADs evoked by stimulation of the right HPC are also observed in the left HPC during theta (above) and SO (below) states. B. Highpass filtered (30Hz) AD signals during theta (above) and SO (below). C. Inter-hippocampal coherence prior to and during ADs across theta and SO states. D. The average inter-hippocampal coherence is significantly higher for ADs evoked during the SO state than during the theta state.

Figure 5: The occurrence and coordination of spontaneous epileptiform events across
Spontaneous epileptiform events are recorded from right and left hippocampi during SO and theta states. Ongoing spectrographic power is plotted at both 1.17Hz (darker line) and 3.67Hz (lighter line) and was used to determine state and alternations of state. Spontaneous epileptiform events during high periods of ~1Hz activity (SO) are more frequent and larger in amplitude range than those occurring during periods of high ~3Hz activity (theta). Inter-hippocampal crosscorrelation of highpass filtered events across SO (darker line) and theta (lighter line). The highest amplitude positive peak appears with a 4ms lag which is always larger in SO than theta. The normalized interhemispheric crosscorrelations for 0 and 4ms lags for all experiments across states shows higher values for the SO state, suggesting that the propagation of these events is facilitated.

Figure 6: Multiprobe profiles and CSDs of spontaneous epileptiform events during SO and theta. Multiprobe profile of spontaneous epileptiform events recorded during (A) SO and (C) theta states. B, D. Expanded traces of events show a reversal point for the slow components of individual events in the proximal aspect of SRad and for fast components at a more distal level of SRad during both SO and theta states. CSD analysis of spontaneous events show an initial and prominent sink in proximal SRad with another later but still prominent sink region across SPyr and SOr.
A
Theta Afterdischarges
L HPC, R HPC, R CTX
Raw

B
Highpass filtered

C
Inter-HPC Coherence
SO AD, Theta AD

D
Inter-HPC Coherence
Theta, SO