Phase shift in the 24-hour rhythm of hippocampal EEG spiking activity in a rat model of temporal lobe epilepsy

David A. Stanley,1* Sachin S. Talathi,1,2,3* Mansi B. Parekh,2 Daniel J. Cordiner,2 Junli Zhou,2 Thomas H. Mareci,5 William L. Ditto,6 and Paul R. Carney1,2,3,4,7,8

1Department of Biomedical Engineering, University of Florida, Gainesville, Florida; 2Department of Pediatrics, University of Florida, Gainesville, Florida; 3Department of Neuroscience, University of Florida, Gainesville, Florida; 4McKnight Brain Institute, University of Florida, Gainesville, Florida; 5Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida; 6Department of Physics and Astronomy, University of Hawai‘i at Mānoa, Honolulu, Hawaii; 7Department of Neurology, University of Florida, Gainesville, Florida; and 8Wilder Center of Excellence for Epilepsy Research, University of Florida, Gainesville, Florida

Submitted 17 October 2012; accepted in final form 10 May 2013

Stanley DA, Talathi SS, Parekh MB, Cordiner DJ, Zhou J, Mareci TH, Ditto WL, Carney PR. Phase shift in the 24-hour rhythm of hippocampal EEG spiking activity in a rat model of temporal lobe epilepsy. J Neurophysiol 110: 1070–1086, 2013. First published May 15, 2013; doi:10.1152/jn.00911.2012.—For over a century, epileptic seizures have been known to cluster at specific times of the day. Recent studies have suggested that the circadian regulatory system may become permanently altered in epilepsy, but little is known about how this affects neural activity and the daily pattern of seizures. To investigate, we tracked long-term changes in the rate of spontaneous hippocampal EEG spikes (SPKs) in a rat model of temporal lobe epilepsy. In healthy animals, SPKs oscillated with near 24-h period; however, after injury by status epilepticus, a persistent phase shift of ~12 h emerged in animals that later went on to develop chronic spontaneous seizures. Additional measurements showed that global 24-h rhythms, including core body temperature and theta state transitions, did not phase shift. Instead, we hypothesized that locally impaired circadian input to the hippocampus might be responsible for the SPK phase shift. This was investigated with a biophysical computer model in which we showed that subtle changes in the relative strengths of circadian input could produce a phase shift in hippocampal neural activity. MRI provided evidence that the medial septum, a putative circadian relay center for the hippocampus, exhibits signs of damage and therefore could contribute to local circadian impairment. Our results suggest that balanced circadian input is critical to maintaining natural circadian phase in the hippocampus and that damage to circadian relay centers, such as the medial septum, may disrupt this balance. We conclude by discussing how abnormal circadian regulation may contribute to the daily rhythms of epileptic seizures and related cognitive dysfunction.

Circadian rhythms are 24-h oscillations that are ubiquitous in biological systems ranging from bacteria to the human brain. In mammals, coordination of circadian rhythms is achieved by the circadian timing system, which consists of the central pacemaker in the suprachiasmatic nucleus (SCN) that drives 24-h oscillations in a number of secondary relay centers (Gerstner and Yin 2010). These relay centers disseminate clock information by synaptic and hormonal signaling, thereby coordinating 24-h rhythms throughout the body.

In many brain regions, neural activity is regulated throughout the day-night cycle. Studies have shown daily variation in neural unit activity (Brunel and de Montigny 1987; Inouye and Kawamura 1979; Munn and Bilkey 2012; Yamazaki et al. 1998), evoked response (Barnes et al. 1977), long-term potentiation (LTP) (Chaudhury et al. 2005), and spike frequency accommodation (Kole et al. 2001). A number of factors may facilitate this modulation, including synaptic and hormonal input from circadian relay centers located both within and outside the hypothalamus (Saper et al. 2005). For example, the pineal gland receives indirect input from the SCN and releases melatonin into the bloodstream at night. Melatonin receptors are dispersed throughout the brain (Mazzucchelli et al. 1996; Musshoff et al. 2002) and in turn modulate GABAergic synaptic transmission (Wan et al. 1999). Likewise, as shall be discussed, the septum is another putative circadian relay center (Ruby et al. 2008) that receives input from the SCN (Morin et al. 1994) and projects to multiple limbic structures (Paxinos 2004). In addition to input from circadian relay centers, intrinsic cellular processes can also drive 24-h rhythms of neural activity (Kole et al. 2001; Liu et al. 2000). In this manner, neural activity is coordinated on a 24-h basis by a variety of mechanisms in order to meet daily demands.

A fundamental question in circadian research is to what extent neurological diseases might affect the circadian system and impair its ability to regulate circadian rhythms. Disrupted circadian organization can have extensive pathological implications, including cognitive and emotional disorders, temporal lobe atrophy, and increased risks for cancer and cardiovascular diseases (Cho 2001; Wulff et al. 2010). For the case of epilepsy, it has been proposed that permanent structural changes in the brain associated with epilepsy may block or impair circadian rhythms (Quigg 2000). This is supported by a number of studies on global markers of circadian rhythms, which have shown abnormalities of the wake-sleep cycle (Bastlund et al. 2005; Shouse et al. 1996), increased variability in core body temperature (CBT) recordings (Quigg et al. 1999), and altered melatonin release (Hofstra and de Weerd 2009; Quigg 2000).

While the above studies on global markers suggest that the circadian system is altered in epilepsy, the functional implications of such alteration on neural activity are largely unstudied.
Disruption of neural activity on circadian timescales is of interest, since cognitive and emotional disorders commonly associated with epilepsy have been previously linked to jet lag and other forms of circadian disruption (Cho 2001; Wulff et al. 2010). Furthermore, the importance of the circadian system in epilepsy is well established through the observed 24-h periodicity of epileptic seizures (Quigg et al. 2000). Altered circadian input to epileptic brain regions may play a direct role in the creation of excitatory-inhibitory imbalances commonly associated with seizures.

In this report, we systematically investigate longitudinal changes in hippocampal 24-h rhythms throughout preinjury, latency, and spontaneously seizing stages of epileptogenesis in a well-studied rat model of chronic limbic epilepsy. First, we report our long-term in vivo monitoring of EEG activity and characterize a phase shift in hippocampal 24-h rhythms. We then describe a detailed biophysical model and how specific structural changes in the circadian system can produce such a phase shift. Finally, using MRI, we demonstrate structural changes in the medial septum, a putative circadian relay such a phase shift. Finally, using MRI, we demonstrate structural changes in the medial septum, a putative circadian relay center, and describe how this could contribute to the observed phase shift. We conclude with a discussion on the implications of our findings for the observed 24-h patterns of seizure recurrence.

MATERIALS AND METHODS

Animal methods. Animal studies were conducted on 2-mo-old male Sprague-Dawley rats weighing 200–265 g. Protocols were approved by the University of Florida’s Institutional Animal Care and Use Committee (IACUC protocol no. D710). All animals were housed in a 24-h symmetrical light-dark environment with light stage centered at 12:00 noon and with constant temperature and humidity levels. Food and water were refilled at regular intervals such that they were continually available, and animal housing was regularly cleaned. Animals were monitored with continuous time-locked video. Injury was induced with a well-characterized model for chronic temporal lobe epilepsy (TLE) (Lothman et al. 1989, 1990; Sanchez et al. 2006), in which electrical induction of status epilepticus (SE) is used to bring about a state of recurrent spontaneous seizures after a latency period of 2–4 wk. Animals were monitored during preinjury, latency, and spontaneously seizing stages of epileptogenesis. The latency period was included in this analysis because spontaneous seizures have been previously shown to promote transient perturbations to circadian rhythms (Quigg et al. 2001). The beginning of the spontaneously seizing stage was marked by the first recorded grade 3 or greater seizure.

A total of \( N = 20 \) animals were divided into three groups for this study: \( N = 6 \) for EEG recording (3 spontaneously seizing, 3 stimulated but nonseizing), \( N = 3 \) for CBT recording (all spontaneously seizing), and \( N = 11 \) for excised MRI (3 control, 8 spontaneously seizing). Of the 8 spontaneously seizing animals used for excised MRI, \( N = 6 \) also provided usable data for longitudinal in vivo imaging. Animals were monitored for at most 60 days.

Because of the experimental demands of continuous longitudinal recording throughout multiple stages of epileptogenesis, it was necessary to use a low number of animals for EEG and CBT rhythm analysis. However, each animal provided many circadian cycles of data (>3 wk per animal), which allowed for clear reconstruction of 24-h rhythms and confident estimation of phase. Additionally, the longitudinal nature of our recordings allowed, for each animal, postinjury data to be compared directly to preinjury data in a paired fashion. A summary of the experimental timeline is supplied in Fig. 1. A and B. Surgical, electrophysiological, data analysis, imaging, and modeling techniques are described below.

Surgical methods. Prior to all surgeries, rats were anesthetized by subcutaneous injection of 10 mg/kg xylazine (Webster Veterinary) and maintained anesthetized with 1.5% isoflurane (Akrone). All rats were stereotaxically implanted in the right ventral hippocampus [5.3 mm caudal to bregma, 4.9 mm lateral (right) of bregma, and 5 mm ventral from dura] with a Teflon-coated bipolar twisted electrode (330-μm diameter), which was later used for induction of SE. Additionally, for collection of EEG data, 16-channel Omnetics-based microwire recording electrode arrays (Tucker Davis Technologies, Alachua, FL) were implanted bilaterally into the dentate gyrus and CA1 regions (Fig. 1C). Electrodes were made of tungsten with polyimide insulation, had 2-mm tip length, and were 50 μm in diameter. The microwire array was anchored to the skull with four 0.8-mm stainless steel screws. Two were AP 2 mm to bregma and bilateral 2 mm, and two were AP –2 mm to the lambda/dural suture and bilateral 2 mm. These two pairs served as the ground and reference, respectively. Electrode placement was verified by MRI after brains had been excised (see below). For animals that underwent MRI and CBT recording, the microwire array was not used and, rather, EEG was monitored directly through the stimulating electrodes. For CBT recording, a radio-frequency TA E-Mitter transponder was inserted in the abdominal cavity and powered by a ER-0000 Energizer/Receiver unit (both from Mini Mitter, Bend, OR) that was placed underneath the cages. This E-Mitter transponder produced significant interference in the hippocampal EEG, and thus EEG data from CBT animals were of poor quality. For this reason, it was necessary to collect EEG and CBT data independently from separate sets of animals.

Induction of self-sustaining status epilepticus. To induce SE, 10-s pulse trains were applied to the bipolar twisted stimulating electrodes with a model 2100 Stimulator (A-M Systems, Sequim, WA), consisting of biphasic square waves with frequency 50 Hz, pulse duration 1 ms, and amplitude 250–400 μA, with 2-s intervals between trains (Lothman et al. 1989, 1990; Sanchez et al. 2006). The protocol for varying the stimulus current amplitude was as follows: The initial stimulus current was 50 μA. This was increased in 25-μA increments either until three consecutive grade 5 seizures were observed or until the upper-limit stimulus current of 600 μA was reached. The stimulation applied to all spontaneously seizing animals fell within the range of 250–400 μA. Animals demonstrated wet dog shakes and seizures throughout the duration of the stimulation procedure, which lasted for 60–90 min. Upon termination of stimulation, EEG recordings documented intermittent self-sustaining 30- to 60-s seizures and interictal 2- to 5-Hz EEG activity lasting for ~24 h. After a latency period of 2–4 wk, rats developed spontaneous recurrent limbic seizures. Recordings of the latency period began 2 days after SE stimulus. For EEG recordings, a total of \( N = 6 \) animals were stimulated. These were divided into two groups: those that exhibited spontaneous seizures (\( N = 3 \)) and those that did not exhibit seizure activity for at least 4 wk after stimulus (\( N = 3 \)). For CBT and MRI analysis, only rats that developed spontaneous seizures were analyzed.

Stimulation was performed between 9:00 AM and 3:00 PM.

Seizure detection. An in-house seizure detection system (Talathi et al. 2008) was used to scan EEG data sets to identify potential seizure epochs. These data sets were then visually scored by an expert epileptologist (P. R. Carney). The Racine grades of the seizures were confirmed from video recordings (Racine 1972). Seizure information was used to assess the beginning of the spontaneously seizing stage of epileptogenesis, which was marked by the first recorded grade 3 or greater seizure. Because of E-Mitter transponder interference, described above, seizures in CBT recording animals were identified exclusively on the basis of behavioral measures. Therefore, we did not attempt to split CBT postinjury data into latency and spontaneously seizing stages.

EEG and CBT data acquisition. The EEG data from the recording electrodes was channeled through a 32-channel commutator to a pair of 16-channel RA16PA Medusa PreAmps with frequency response between 1.5 Hz and 7.5 Hz (Tucker Davis Technologies). The EEG
signal was digitized at the rate of 12 kHz and passed onto the digital signal processing (DSP) unit, the Pentusa RX-5 acquisition board (Tucker Davis Technologies). The data from the DSP unit were streamed onto a PC and stored for further processing. CBT data were streamed through the ER4000 receiver and stored on a PC with Vital View software (Mini Mitter) at a sampling rate of one data point per second.

Spike detection. From EEG data, we extracted large-amplitude EEG events, which included both hippocampal sharp waves (SPWs) and interictal spikes (IS) (Buzsáki et al. 1983; Suzuki and Smith 1987). We shall refer to SPWs and IS collectively as hippocampal EEG spikes (SPKs). Our procedure for identifying and sorting SPKs was detailed previously (Talathi et al. 2009). Briefly, data from a single microwire channel in CA1 were divided into 1-h nonoverlapping epochs. High-amplitude events were detected when the signal exceeded a threshold of $5\sigma$, where $\sigma$ is the standard deviation of the data in the epoch. Events were centered and normalized within a 0.45-s window and were then input into a customized version of an established spike clustering algorithm (Fee et al. 1996). This algorithm ensured that spikes with consistent waveform were tracked throughout the experiment (Fig. 2).

Theta epoch detection. Theta activity in the hippocampus is distributed throughout the wake-sleep cycle. Given the fact that theta state transitions can affect the occurrence of many hippocampal activities, including SPWs, IS, and gamma rhythms (Buzsáki et al. 1991; Leung 1988; Suzuki and Smith 1987), we sought to quantify the distribution of theta activity throughout the day. Epochs of hippocampal theta activity were identified with an automated routine (Belluscio et al. 2012; Csicsvari et al. 1998). We calculated the ratio of the power in theta (6–10 Hz) to the power in delta (1–6 Hz) in 2-s epochs and classified data as predominantly theta when this ratio exceeded a specified threshold. The threshold was 2.0 by default, although the sensitivity of our results to a range of threshold values between 1.0 and 3.0 in steps of 1.0 was examined (see APPENDIX, Table A1). Similarly, our results were found to be insensitive to other common choices of theta and delta bands, such as 5–10 Hz and 2–4 Hz, respectively.

EEG and CBT 24-h rhythm phase analysis. For purposes of analyzing 24-h rhythms in SPK rate, theta epoch occurrence, and CBT time series, all time series were treated in a similar manner, similar to that described previously (Talathi et al. 2009). First, time series data were pooled into 1-h nonoverlapping time bins and the average value for each 1-h time bin was calculated. Data were then smoothed using 6-h, 90%-overlapping moving average windows. We observed that, for some time series, data values would drift over the course of days or weeks. This was particularly the case for SPK rates, as was previously investigated (Talathi et al. 2009), and we refer to this as baseline drift. To detrend data by removing these long-timescale changes, we estimated the baseline drift by averaging data within a 1-day moving window. Then, we subtracted these baseline values...
from the original data. In figures where detrended (baseline subtracted) data are shown, we use the symbol $\Delta$ to denote that the plotted value is a deviation from baseline. We also explored the use of alternative window sizes and overlap values for smoothing and baseline calculations and found that these changes had minimal effect on the results (see Appendix, Fig. A1).

To obtain phase information, the smoothed time series data were then compressed into a single 24-h time window. This was achieved by applying the mapping $T_n \rightarrow T_n$ modulo 24 h, where $T_n$ is the time point in hours associated with each data value $X_n$ in the smoothed time series. Data were then fitted to sinusoidal functions of the form $f = A \times \cos(2\pi(T - T_0)/24)$ with least-squares minimization. To confirm that the data were indeed sinusoidal, we conducted the zero-amplitude test (Nelson et al. 1979). We identified one nonseizing animal for which the null hypothesis of zero amplitude could not be rejected at $P < 0.05$ ($P = 0.052$), and thus we removed this animal from the study. This reduced the number of nonseizing animals from $N = 3$ to $N = 2$. The 95% confidence intervals associated with phase estimates were estimated as previously described (Nelson et al. 1979).

All statistical tests reported are paired-samples $t$-tests unless otherwise specified. In all cases, significance is considered to be $P < 0.05$. Error bars represent SE unless otherwise stated.

**MRI data collection and analysis.** For MRI, the rats were continuously video/EEG recorded for a period of 60 days post-SE. EEG monitoring was performed through the stimulating electrodes. Temporal changes in the rat brains were monitored in vivo with MRI pre-SE and also at days 3 and 60 post-SE at 11.1 T. The rats were initially anesthetized with 4% isoflurane in 2.0 l/min O$_2$, and then 4 mg/kg of xylazine was injected subcutaneously, along with 2 ml of lactated Ringer solution (Hospira, Lake Forrest, IL) to maintain the physical condition of the rats during the extended MRI scans. Each rat was placed in a prone position, in a custom-made MRI-compatible stereotaxic frame and cradle, to allow repeatable positioning and minimize motion artifacts. In the magnet, anesthesia was maintained with 1.5–2.0% isoflurane in O$_2$ at 1 l/min. Respiration and temperature were monitored, and physiological temperature was maintained with heated air flowing over the animal (SA Instruments, Stony Brook, NY). Magnetic resonance was measured with a custom-built, saddle-shaped 470-MHz coil, for both excitation and detection, positioned on top of the rat’s head and centered over the brain. In vivo acquisition parameters for diffusion-weighted imaging (DWI) and T2 measurements have previously been published (Parekh et al. 2010). DWI data were collected in 27 directions with a b value of 800 s/mm$^2$ and in 6 directions with a b value of 100 s/mm$^2$ and were then fitted to a rank-2 tensor to calculate average diffusivity (AD) and fractional anisotropy (FA). A region of interest was manually drawn for the hippocampus to calculate average diffusivity (AD) and fractional anisotropy (FA). A region of interest was manually drawn for the hippocampus to calculate average diffusivity (AD) and fractional anisotropy (FA). A region of interest was manually drawn for the hippocampus to calculate average diffusivity (AD) and fractional anisotropy (FA). A region of interest was manually drawn for the hippocampus to calculate average diffusivity (AD) and fractional anisotropy (FA). A region of interest was manually drawn for the hippocampus to calculate average diffusivity (AD) and fractional anisotropy (FA). A region of interest was manually drawn for the hippocampus to calculate average diffusivity (AD) and fractional anisotropy (FA).

**CA3 network model.** Our in vivo analysis tracked 24-h oscillations in the rate of spontaneous hippocampal SPKs. The SPKs recorded experimentally in CA1 are known to originate as a result of synchronous population bursts in CA3 circuitry (Buzsáki 1986). Therefore, modeling efforts were focused on circadian regulation of neural activity in region CA3. The network model contained 200 CA3 pyramidal cells, 25 basket cells, and 25 stratum oriens interneurons that project into the lacunosum moleculare (O-LM). In addition, to represent circadian input from the medial septum, we also included a population of medial septal GABAergic (MSG) neurons. We focused specifically on septal GABAergic rather than septal cholinergic input because pharmacological (Buzsáki 1986; Suzuki and Smith 1988b) and lesioning (Lee et al. 1994) studies have shown little cholinergic effect on SPK rates in vivo. The pyramidal neuron model was comprised of 19 compartments, with 8 compartments for the basal dendrites, 10 for the apical dendrites, and a single somatic compartment (Traub et al. 1991). Each compartment contained active and passive channel conductances (Hodgkin and Huxley 1952) and was connected to other compartments by passive resistances (Rall 1962). All interneurons were single compartmental. For all neuron types, intrinsic ionic currents and biophysical parameters in each compartment were obtained from previous models (Traub et al. 1991; Wang 2002; Wang and Buzsáki 1996). Default current injection values for pyramidal, basket, O-LM, and MSG were 500 pA, 0 pA, −10 pA, and 21 pA, respectively. Simulations were run in GENESIS 2.3 (Bower and Beeman 2003; Bower et al. 2002) on a Mac XServ cluster, and data were analyzed in MATLAB R2011a. Code for interneuron cell types was obtained from a previous implementation (Hajós et al. 2010).
Synaptic time constants were those used previously (Neymotin et al. 2011; Taxidis et al. 1999; Traub and Miles 1991), where available, as well as on experimentally measured unitary excitatory/inhibitory postsynaptic potential values (Traub et al. 2004), available on ModelDB with accession number 142104.

Network properties. The CA3 network properties were specified in terms of synaptic strengths, synaptic time constants, and synaptic connectivity. Synaptic currents were implemented with the standard double-exponential mechanism, using the GENESIS synchan object (Bower and Beeman 2003). Pyramidal cells formed AMPA synapses, while all interneurons formed GABA_A synapses. Table 1 lists synaptic strengths, specifying the maximal conductance of a given synaptic connection. These values were based on experimentally measured unitary excitatory/inhibitory postsynaptic potential values (Traub et al. 1999; Traub and Miles 1991), where available, as well as on previous models (Hajós et al. 2004; Neymotin et al. 2011; Taxidis et al. 2012). Synaptic time constants were those used previously (Neymotin et al. 2011), with AMPA synapses having \( \tau_1 = 0.05 \text{ ms} \) and \( \tau_2 = 5.3 \text{ ms} \). All GABAergic synapses had \( \tau_1 = 0.07 \text{ ms} \) and \( \tau_2 = 9.1 \text{ ms} \), with the exception of synapses with presynaptic O-LM cells. In such cases, \( \tau_1 = 0.2 \text{ ms} \) and \( \tau_2 = 20 \text{ ms} \) (Neymotin et al. 2011). A schematic diagram of the network connectivity is shown in Fig. 3, and in Table 2 we report network connectivity in terms of the number of presynaptic neurons converging on each postsynaptic neuron. Connectivity values were derived from paired recordings of presynaptic and postsynaptic cells (Traub and Miles 1991) as well as from previous modeling studies (Hajós et al. 2004; Neymotin et al. 2011; Taxidis et al. 2012; Traub et al. 1996). For example, the connection probability from CA3 excitatory cells to CA3 stratum pyramidale inhibitory cells (\( p_{\text{pyr2inc}} \)) was experimentally measured as 0.1 (Traub and Miles 1991), and therefore synaptic convergence of pyramidal cells onto basket cells in our network was set to give \( N_{\text{pyr}} \times p_{\text{pyr2inc}} = 200 \times 0.1 = 20 \). Pyramidal cells received innervation from other pyramidal cells in the basal dendrites (4th basal compartment from the soma). Basket cells innervated the soma of pyramidal cells, whereas O-LM cells innervated the distal apical dendrites (9th apical compartment from the soma).

Background activity. The background network activity was modeled by Poisson-distributed excitatory and inhibitory postsynaptic potentials. These synapses impinged on the soma of pyramidal cells and interneurons and also on the most distal apical dendritic compartment of pyramidal cells. The mean frequency of AMPA and GABA background events was 1,000 Hz, and the maximal background synaptic conductances are provided in Table 3. These values were chosen to provide symmetrical resting membrane potential fluctuations at \(-65 \text{ mV} \) with standard deviations between 1 and 2 mV (Destexhe et al. 2003).

Circadian modulation. To represent circadian drive, we incorporated three major inputs to the model that were subject to 24-h modulation (Fig. 3). These inputs were defined as a function of the 24-h circadian time variable, \( T \), with \( T = 0 \text{ h} \) corresponding to midnight and \( T = 12 \text{ h} \) to noon. Since circadian changes happen on much slower timescales compared with the neural network’s dynamics, a total of 16 separate simulations were run, in which \( T \) was varied as a model parameter. Each simulation produced 10 s of data, and the first second was discarded prior to analysis in order to remove transient effects.

The first circadian input was modulation of the medial septum. Multiunit recordings have shown that medial septum activity possesses a robust circadian rhythm, peaking at night (Yamazaki et al. 1998). Modulation of the medial septum was achieved by scaling the tonic current injection, \( I_{\text{inj}} \), to MSG cells: \( I_{\text{inj}} = I_{\text{inj}} \times S_{\text{septal}} \). In this equation, \( I_{\text{inj}} \) is the default current injection for MSG cells, as described above, \( S_{\text{septal}} = \left[ 1 + \text{C}_{\text{septal}} \cos \left( 2\pi T / 24 \right) \right] \) is the sepal circadian scaling factor, and \( \text{C}_{\text{septal}} = 0.25 \) is the sepal circadian scaling coefficient. This input caused maximal sepal activity to peak when \( T = 0 \text{ h} \). In our model, we focused specifically on sepal activity.

### Table 1. Maximal synaptic conductances

<table>
<thead>
<tr>
<th>Synapse</th>
<th>Pyramidal</th>
<th>Basket</th>
<th>O-LM</th>
<th>MSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr</td>
<td>2.6</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Bsk</td>
<td>9.2</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-LM</td>
<td>8.3</td>
<td></td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>MSG</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Values are in nS. Pyr, pyramidal cell; basket, basket cell; O-LM, stratum oriens interneuron projecting into lacunosum moleculare; MSG, medial septal GABAergic interneuron.

### Table 2. CA3 network connectivity expressed in terms of synaptic convergence

<table>
<thead>
<tr>
<th>Synapse</th>
<th>Pyramidal</th>
<th>Basket</th>
<th>O-LM</th>
<th>MSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr (N = 200)</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Bsk (N = 25)</td>
<td>15</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-LM (N = 25)</td>
<td>10</td>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>MSG (N = 25)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

Traub et al. (1996). For example, the connection probability from CA3 excitatory cells to CA3 stratum pyramidale inhibitory cells (\( p_{\text{pyr2inc}} \)) was experimentally measured as 0.1 (Traub and Miles 1991), and therefore synaptic convergence of pyramidal cells onto basket cells in our network was set to give \( N_{\text{pyr}} \times p_{\text{pyr2inc}} = 200 \times 0.1 = 20 \). Pyramidal cells received innervation from other pyramidal cells in the basal dendrites (4th basal compartment from the soma). Basket cells innervated the soma of pyramidal cells, whereas O-LM cells innervated the distal apical dendrites (9th apical compartment from the soma).

### Table 3. Background synaptic activity conductances

<table>
<thead>
<tr>
<th>Cell</th>
<th>Synapse</th>
<th>Section</th>
<th>Conductance, nS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyramidal</td>
<td>AMPA</td>
<td>Soma</td>
<td>1.0</td>
</tr>
<tr>
<td>Pyramidal</td>
<td>AMPA</td>
<td>Dendrite</td>
<td>1.0</td>
</tr>
<tr>
<td>Basket</td>
<td>AMPA</td>
<td>Soma</td>
<td>0.03</td>
</tr>
<tr>
<td>O-LM</td>
<td>AMPA</td>
<td>Soma</td>
<td>0.03</td>
</tr>
<tr>
<td>Pyramidal</td>
<td>GABA_A</td>
<td>Soma</td>
<td>2.5</td>
</tr>
<tr>
<td>Pyramidal</td>
<td>GABA_A</td>
<td>Dendrite</td>
<td>2.5</td>
</tr>
<tr>
<td>Basket</td>
<td>GABA_A</td>
<td>Soma</td>
<td>0.01</td>
</tr>
<tr>
<td>O-LM</td>
<td>GABA_A</td>
<td>Soma</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Fig. 3. Network connectivity in the neural network model. Sites of circadian input to region CA3 and to the medial septum are indicated by 

Additionally, all GABAergic synapses are subject to circadian modulation by melatonin. BC, basket cells; MSG, medial septal GABAergic interneurons; O-LM, stratum oriens interneurons projecting into lacunosum moleculare; PYR, pyramidal neurons.
GABAergic input because, as mentioned above, previous studies have shown little cholinergic effect on SPK rates in vivo (Buzsáki 1986; Lee et al. 1994; Suzuki and Smith 1988b).

Second, melatonin, a primary systemic output of the circadian system, is released nightly by the pineal gland. In the hippocampus, melatonin has an excitatory effect and decreases the strength of GABAergic inhibition (Wan et al. 1999). To account for the effects of melatonin, all GABAergic maximal synaptic conductances (including background synapses) were multiplied by the scaling factor $S_{\text{mel}} = 1 + C_{\text{mel}} \cos(2\pi(T - 12)/24)$, producing maximum attenuation of GABA at night. $C_{\text{mel}} = 0.10$, with the exception of Fig. 7E, where $C_{\text{mel}}$ is varied across a range of parameters (described below).

Finally, a variety of other factors can also influence CA3 activity, including 24-h variation of CA3 pyramidal neuron calcium currents (Kole et al. 2001), adenosine release (Liu et al. 2000), and neuro-modulatory effects. As a simplifying assumption, we represented the combined effects of these additional circadian inputs by sinusoidal modulation of pyramidal cell current injection. Experimental studies suggest that 24-h modulation of CA3 pyramidal cell firing activity peaks during the day (Brunel and de Montigny 1987), and therefore pyramidal current injection was modulated as follows: $I_{\text{inj}} = I_{\text{inj}}^0 S_{\text{pyr}}$, where $I_{\text{inj}}^0$ is the default current injection for pyramidal cells, as described above, $S_{\text{pyr}} = 1 + C_{\text{pyr}} \cos(2\pi(T - 12)/24)$, and $C_{\text{pyr}} = 0.25$.

The default strengths of the circadian scaling coefficients $C_{\text{septal}}$, $C_{\text{pyr}}$, and $C_{\text{inj}}$ were based on experimental studies where available. Specifically, $C_{\text{mel}} = 0.10$ was chosen to produce an $\sim 20\%$ change in GABA$_A$ currents, consistent with that observed upon application of physiological levels of melatonin in slice (Wan et al. 1999). $C_{\text{pyr}}$ was constrained so that pyramidal cell firing peaked during the day, in order to ensure agreement with experimental measurements (Brunel and de Montigny 1987), although we note that this experimentally measured daytime peak showed a seasonal dependence as well. $C_{\text{septal}}$ was chosen such that interneurons were entrained to septal circadian drive. Thus, while $C_{\text{mel}}$ was constrained by direct experimental measurement, $C_{\text{pyr}}$ and $C_{\text{septal}}$ were adjusted as parameters to enable the model to reproduce functional activity.

**Sensitivity analysis.** To evaluate the sensitivity of our modeling results to variation in the relative strengths of circadian inputs, we swept melatonin and septal circadian inputs through a range of values. The strength of the melatonin circadian input was varied by sweeping $C_{\text{mel}}$, between 0.05 and 0.18. The strength of medial septal innervation was varied by randomly disabling MSG cells, so as to simulate injury to the septum. The percentage of remaining MSG cells, $I_{\text{MSG}}$, was varied between 0 and 100%. All other parameters were held at their default values. For each $(C_{\text{sept}}, I_{\text{MSG}})$ pair of values, a set of simulations were run in which median time $T$ was varied and the resulting firing rate data were fit to a sinusoid with 24-h period to estimate acrophase. For some $(C_{\text{sept}}, I_{\text{MSG}})$ pairs, sinusoids could not be reliably fit to the data. Generally, these instances corresponded to situations in which the relationship between firing rate and time was either nonsinusoidal or of very low amplitude. Therefore, we rejected fits for which the mean squared error (MSE) was $> 33\%$ of the data’s variance and for which the amplitude of the sinusoidal oscillation was $< 3\%$ of the mean firing rate.

We also investigated the sensitivity of the model to changes in its internal network connectivity. Drastic network changes could alter the firing activity patterns produced by the network. Subtle network changes could affect how 24-h rhythms propagated throughout the network. However, in most cases, the network was stable for changes in convergence values by $\pm 5$ neurons. Additionally, greater changes in convergence values could be accommodated by adjusting $C_{\text{pyr}}$ and $C_{\text{septal}}$, which we tuned as free parameters.

**RESULTS**

Phase shifting of hippocampal neural activity occurs relative to daily rhythms of core body temperature and hippocampal theta activity. In vivo EEG recordings were performed continuously throughout the preinjury, latency, and chronic stages of the TLE model. From this data, we extracted spontaneous large-amplitude EEG events or SPKs (Fig. 2, MATERIALS AND METHODS), which included both SPWs and IS (Buzsáki et al. 1983; Suzuki and Smith 1987). Together, SPWs and IS have previously been used to quantify 24-h rhythms of hippocampal neural activity (Talathi et al. 2009). Because of their generation within the hippocampus (Buzsáki 1986) and the similarities in their underlying mechanisms of generation (Buzsáki et al. 1991; Leung 1988; Suzuki and Smith 1987), they are an ideal marker for spontaneous neural activity originating within the hippocampus.

SPKs were tracked continuously throughout the day in each animal and analyzed longitudinally. SPK rates in each animal showed distinct 24-h oscillations (Fig. 4A). However, after injury, a phase shift emerged of $\sim 12$ h (Fig. 4B). This phase shift was statistically significant and persisted throughout the latency period ($P = 0.010, N = 3$ rats) and after the advent of spontaneous seizures (Fig. 4, C and D) ($P = 0.0078, N = 3$ rats).

Going from preinjury to latency periods, the phase shifts experienced by these three animals were $11.9 \pm 1.4, 9.1 \pm 1.1$, and $9.0 \pm 0.7$ h (mean $\pm 95\%$ confidence). A similar phase shift was reported previously (Talathi et al. 2009); however, the origin of the phase shift was not clear, and, furthermore, it was not clear whether the phase shift was related to the emergence of seizures. Therefore, we also analyzed SPK rates in animals that were stimulated into SE but did not successfully exhibit chronic spontaneous seizures. These animals were monitored for at least 4 wk after initial SE to confirm the absence of seizures. We found that, although there was some drift in the phase, it was much less severe than for the seizing animals (Fig. 4, E and F). Specifically, the phase shifts observed for the two nonseizing animals were $5.6 \pm 2.3$ and $2.5 \pm 1.5$ h (mean $\pm 95\%$ confidence). These data suggest that the phase shift is correlated with the emergence of seizures following SE stimulation; however, further tests need to be done to determine whether the phase shift plays a causal role in the emergence of seizures.

While the aforementioned analysis was based on a low sample size ($N = 3$ seizing, $N = 2$ nonseizing animals), the results were consistent in that each of the three seizing animals showed a phase shift between 9 and 12 h. Since phases were estimated over many 24-h cycles from baseline-subtracted data, it was possible to obtain highly confident estimates of phase for each animal; the $95\%$ confidence intervals associated with acrophase estimates were at most $\pm 1.3$ h for seizing animals, and all reported animals passed the zero-amplitude test with $P < 0.0001$ (Nelson et al. 1979).

Given that the master circadian clock in the SCN plays a central role in entraining 24-h rhythms throughout the body, we tracked daily rhythms of CBT, a well-validated biomarker for SCN rhythms (Hofstra and de Weerd 2008). Overall, the phase of CBT circadian rhythms did not shift significantly during the epileptogenic period (Fig. 5) ($P = 0.68, N = 3$ rats),

---

1 This phase shift persisted throughout the entire postinjury stage. Persistence of the phase shift is reported in the APPENDIX (see Fig. A2).
although the variability in the signal did increase, as has been previously reported (Quigg et al. 1999). This suggests that the SPK phase shift cannot be attributed to a phase shift in the SCN master circadian clock.

Perturbations to activity rhythms and sleep architecture have been reported in animal and human epilepsy (Bastlund et al. 2005; Shouse et al. 1996; Stewart and Leung 2003) and might also contribute to the SPK phase shift. Activity and sleep state

![Diagram](http://www.jn.org/jnpho.png)

Fig. 4. Phase shift in the 24-h rhythm of spontaneous hippocampal SPKs. A and B: chronic tracking of SPK rates for Pre, Post-L, and Post-SS stages of epileptogenesis for a single rat. Days are marked from the start of preinjury recording in A and from the day of status epilepticus (SE) in B. Dot-dashed vertical lines correspond to 0000 (midnight). The gaps in the traces reflect data missing because of technical problems. C: the baseline drift in SPK rate was subtracted out, and the resulting detrended time series was combined into a 24-h time window (modulo 24). This was done for Pre, Post-L, and Post-SS stages of epileptogenesis. Cosine fits (gray) reveal a postinjury phase shift. D: estimating the average time of minimum SPK activity across all animals showed a statistically significant phase shift during Post-L and Post-SS stages. The time of minimum SPK activity was measured to avoid the discontinuity between 2359 and 0000. E: SPK rates from a rat that was stimulated into SE but did not successfully develop spontaneous seizures after 4 wk of monitoring. Preinjury (Pre) and postinjury (Post) stages are shown. Cosine fits (gray) show a slight drift in phase following injury. F: for animals that did not develop spontaneous seizures, the postinjury phase shift did not reach significance. Values are means ± SE. *P < 0.05 by t-test.

![Diagram](http://www.jn.org/jnpho.png)

Fig. 5. Circadian phase of CBT is unper- turbed after injury. A and B: tracking of 24-h rhythms of CBT is shown for a single spontaneously seizing rat before and after injury, respectively. Days are marked from the start of preinjury recording in A and from the day of SE in B. Note that since CBT recordings did not include simultaneous EEG because of interference from the transponder, we did not attempt to split postinjury data into latency and spontaneously seizing stages. C: data were compressed into single 24-h time windows as in previous figures. There was no observed phase shift after injury, although the variability of the signal did increase. D: the lack of phase shift in CBT activity after injury was consistent across rats examined. The time of minimum CBT activity was measured to avoid the discontinuity between 2359 and 0000. Values are means ± SE.
can influence SPKs by affecting the transition of the hippocampus between theta (activated) and non-theta (deactivated) states (Buzsáki 1996). The theta state occurs during awake mobility and rapid eye movement (REM) sleep, while the non-theta state occurs during awake immobility and slow-wave sleep (Kramis et al. 1975). It is well established that SPWs are absent during theta and that IS rates are substantially reduced during theta (Buzsáki et al. 1991; Leung 1988; Suzuki and Smith 1987). Therefore, suppression of SPKs can emerge at different times of day resulting from activities that promote hippocampal theta.

To investigate whether hippocampal theta is altered in such a way that could affect the daily pattern of SPKs, we measured changes in the average time spent in the theta state throughout the day-night cycle. Theta was distinguished from non-theta on the basis of the ratio between the power in the theta band (6−10 Hz) and the power in the delta band (1−6 Hz) in 2-s epochs of data (Belluscio et al. 2012; Csicsvari et al. 1998) (see MATERIALS AND METHODS). Example traces of each EEG state are shown in Fig. 6, A and B. We observed that the distribution of theta activity over the day-night cycle showed a 24-h rhythm that did not shift phase after injury (Fig. 6, C−E), a result that was consistent across all rats examined (Fig. 6F) (*P = 0.71 and P = 0.31 for latency and spontaneously seizing stages, respectively; N = 3 rats). This suggests that the SPK phase shift is not driven by a phase shift in the daily distribution.

Fig. 6. Interaction of SPKs with the 24-h rhythm of theta activity cannot explain SPK phase shift. A and B: extracted 2-s epochs of theta activity and non-theta, respectively. SPK event is indicated by arrow. Ratio of theta to delta power is 3.28 in theta (A) and 0.09 in non-theta (B). C and D: fraction of time spent in theta state for Pre, Post-L, and Post-SS stages of epileptogenesis for a single rat. Days are marked from the start of preinjury recording in C and from the day of SE in D. The gaps in the traces reflect data missing because of technical problems. E: the baseline drift in theta activity was subtracted from the data shown in C and D, and the resulting detrended time series was compressed into a 24-h time window. The theta activity trends show a 24-h rhythm that does not shift phase between Pre, Post-L, and Post-SS stages. Cosine fits are indicated by solid lines. F: examining the time of minimum theta activity across all rats showed that there was no significant change in the theta rhythm’s 24-h oscillation. G: examination of SPKs exclusively in the non-theta state allowed for tracking of SPK rates independent of transitions between theta and non-theta states. As before, a phase shift was observed after injury. H: this analysis was repeated for all rats, and we observed a statistically significant phase shift between the preinjury and postinjury stages of epileptogenesis. Values are means ± SE. *P < 0.05 by t-test.
of hippocampal theta. Similar findings were obtained when varying the ratio for theta detection between 1.0 and 3.0 (see Appendix, Table A1).

SPKs may include both SPWs and IS, and, as discussed above, while SPWs are absent during theta, IS can occur during theta states (Buzsáki et al. 1991; Leung 1988; Suzuki and Smith 1987). Therefore, we hypothesized that the emergence of IS could contribute to the phase shift by promoting the emergence of SPKs during times of day when theta is prevalent. To investigate this, we tracked the rate of SPKs occurring exclusively in the non-theta state (Fig. 6, G and H). A phase shift of ~12 h appeared, a result that was consistent across all rats examined (P = 0.0073 and P = 0.00094 for latency and spontaneously seizing stages, respectively; N = 3 rats). This suggests that the phase shift is not dependent on IS occurring during the theta state and, in general, is not due to the occurrence of theta state transitions.

Altered balance in circadian input to hippocampus can produce phase shift in a biophysical network model. Given that the circadian phases of CBT and hippocampal theta activity rhythms were found to be stationary, we proceeded to investigate other mechanisms that could account for the SPK phase shift. As discussed above, the circadian system is organized into a hierarchy of relay centers. While the SCN itself does not project directly to the hippocampus, the hippocampus receives circadian input from many other relay centers. It is possible that permanent damage to these centers, as has been hypothesized to occur in multiple types of epilepsy (Quigg 2000), could contribute to the experimentally observed phase shift.

To investigate this hypothesis, we implemented a detailed computer model of circadian regulation in a hippocampal neural network. Three types of hippocampal neurons were modeled, namely, pyramidal cells, basket cells, and O-LM cells (Fig. 7A). In addition, we included a population of MSG neurons. The model’s parameters and network connectivity were based on the CA3 network (Fig. 3), which controls the initiation of CA1 SPKs (Buzsáki 1986; Ellender et al. 2010).

Representation of circadian input in the model was based on the assumption that multiple sources of circadian drive interact in the hippocampus to produce an overall 24-h rhythm. For example, it was recently shown that daily variations in CA1 evoked responses are altered but not eliminated by melatonin knockout (Chaudhury et al. 2005), suggesting the overlapping influence of multiple circadian factors. Three circadian inputs were modeled: circadian modulation of medial septal neural activity, nighttime release of melatonin, and diurnal modulation of hippocampal pyramidal cells (Fig. 7B; see Materials and Methods). These inputs are based on known physiology, and the model was validated to reproduce findings from several experimental studies: rhythmic cycling of pyramidal cell firing rate, peaking during the day (Brunel and de Montigny 1987); increased nighttime inhibitory drive following SE injury (Matzen et al. 2012); and also increased neural activity in response to elevated melatonin (Mushhoff et al. 2002).

When all three circadian inputs were included, pyramidal, O-LM, and basket cell firing rates oscillated in phase, peaking at noon (Fig. 7C). To represent injury, we explored the effects of removing one of the circadian inputs, specifically, the input from MSG cells. This caused basket cell firing acrophase to shift to the night, while pyramidal still peaked at noon (Fig. 7D). Pyramidal cell oscillations also showed an increase in amplitude, resulting in a clear phase misalignment with basket cells. O-LM cells still peaked weakly at noon, but they too could exhibit a weak nighttime peak with increased melatonin drive. To further investigate the origins of the basket cell phase shift, we systematically varied the strengths of both septal and melatonin inputs (Fig. 7E).

$$S_{pyr}$$, $$S_{septal}$$, and $$S_{mel}$$ vary the ratio for theta detection between 1.0 and 3.0 (see Materials and Methods). Each input was simulated by adjusting its respective scaling factor, $$S_{pyr}$$, $$S_{septal}$$, and $$S_{mel}$$ over the 24-h cycle as shown. C and D: simulations of neural activity before (C) and after (D) the complete removal of medial septum input show a 180° phase shift in basket cell 24-h rhythms. MSG cells are indicated by dashed line. E: basket cell firing acrophase is a function of the percentage of septal innervation, $$\delta_{MSG}$$ and the melatonin circadian scaling coefficient, $$C_{mel}$$ (see Materials and Methods). Crosses mark simulation configurations in C and D; regions where sinusoids cannot be reliably fitted for phase estimation are shaded white. For a phase shift to occur, medial septal input must be sufficiently damaged such that the white boundary region is crossed.
vertically (not shown). In general, in order for the basket cell phase shift to occur, the septal circadian input must dominate in the healthy state and then be sufficiently damaged such that the boundary between these two phase-regimes is crossed. The thinness of the boundary region implies sensitivity to small changes in circadian drive; in some cases even an ~20% reduction in septal innervation was sufficient to produce a phase shift, an amount that is comparable with cell loss reported in other parahippocampal regions (Gorter et al. 2003). Additional examination of model dynamics in response to individual circadian inputs is presented in the Appendix (see Fig. A3). Together, these results show that phase shifting of hippocampal neural activity can emerge after a change in the balance of circadian inputs. As we discuss below, this phase shifting of neural firing rates might have direct relevance for explaining the phase shift in experimentally measured SPKs, and might also be relevant for the determining the daily timing of epileptic seizures.

**Structural changes in medial septum and fimbria following injury.** Our modeling work has suggested that changes in the strength of circadian inputs can produce a phase shift in hippocampal 24-h rhythms, even when the phases of the individual circadian inputs remain constant. Such altered circadian drive could arise from damage to circadian relay centers, as has been proposed to occur in epilepsy (Quigg 2000). There are a number of circadian relay centers that could be damaged so as to contribute to this phase shift. We chose specifically to examine the medial septum for signs of structural change for a number of reasons. First, it was recently proposed based on cognitive studies that the septum could be an important circadian relay center between the SCN and the hippocampus (Ruby et al. 2008). Multiunit recordings have shown that the septum possesses a robust circadian rhythm that persists in the absence of external light/dark cues (Yamazaki et al. 1998). Furthermore, it is well established from tracing studies that the septum, unlike the hippocampus, receives heavy innervation from the SCN (Morin et al. 1994). In turn, the medial septum is perhaps the most critical subcortical input to the hippocampus, and lesioning studies have shown that the septum strongly influences the rate of spontaneous generation of SPKs (Buzsáki 1986; Suzuki and Smith 1988a). Given that the medial septum exhibits both circadian rhythmicity and the ability to regulate hippocampal SPKs, we proceeded to investigate anatomical changes in the medial septum.

To study anatomical changes in the medial septum, we performed a longitudinal structural characterization with high-field diffusion- and T2-weighted MRI. From diffusion-weighted MRI data, we calculated measures of AD and FA, which provide information about the magnitude and directionality of molecular displacement, respectively (Pierpaoli et al. 1996). In vivo MRI of the medial septum showed a statistically significant increase in AD and a reduction in T2 relaxometry at 60 days post-SE (Fig. 8A). The increase in AD is suggestive of cell loss, and the reduced T2 likely reflects the sequestering of iron by microglia and astrocytes, which form scars in response to neuronal damage (Zatta 2003). Changes were also seen at 3 days post-SE, reflecting local edema. While these changes do not reach significance, histological studies in a similar animal model have confirmed early cell loss in the septal areas following SE (Gorter et al. 2001, 2003). Since the medial septum projects to the hippocampus primarily by way of the fimbria (Peterson et al. 1987), we performed fiber tracking analysis of the fimbria based on excised diffusion-weighted images. This analysis revealed a statistically significant 32.2 ± 9.5% reduction in fiber volume 60 days post-SE (Fig. 8B). The changes observed in the medial septum (Fig. 8A) imply that this loss of

![Fig. 8. Structural changes in the medial septum and fimbria in epileptogenic animals. A: quantified in vivo average diffusivity (AD), fractional anisotropy (FA), and T2 relaxation values for the medial septum in preinjury, 3 days post-SE, and 60 days post-SE stages for N = 6 rats. Statistically significant changes in AD and T2 are indicative of neuron loss in the medial septum. *p < 0.05, relative to preinjury measurement; #p < 0.05, relative to 3 days measurement. B: fiber tracking of the fimbria in excised control and spontaneously seizing rat brains 60 days post-SE. A significant reduction in total fiber volume is observed in the seizing rats (N = 8 rats) compared with control rats (N = 3 rats). *p < 0.05, unpaired t-test relative to control measurement. Values are means ± SE.](image-url)
fiber volume can be partially accounted for by Wallerian degeneration of medial septal projections to the hippocampus (Pierpaoli et al. 2001). These findings suggest that loss of circadian input to the hippocampus might emerge through degeneration of the medial septum.

**DISCUSSION**

**Summary of results.** We have shown that there emerges a phase shift of ~12 h in the 24-h rhythm of hippocampal spikes in an animal model of limbic epilepsy. This phase shift was evident in all seizing animals examined and was severely reduced in animals that were stimulated but did not develop spontaneous seizures. We measured this phase shift relative to both the CBT rhythm and the 24-h rhythm of hippocampal theta activity and found that neither of these rhythms exhibited a phase shift sufficient to account for the changes in hippocampal SPKs. Rather, motivated by the hypothesis that damage to neuronal pathways might alter circadian rhythms in epilepsy (Quigg 2000), we constructed a detailed biophysical model and showed how changes in circadian inputs to the hippocampus could translate into a phase shift in the 24-h rhythm of neural firing. Finally, we identified a possible structural correlate for this altered circadian input by providing evidence for structural damage to the medial septum, a putative circadian relay center.

While many studies have examined the 24-h rhythm of epileptic seizures in limbic epilepsy (Hofstra and de Weerd 2009; Loddenkemper et al. 2011; Quigg 2000), relatively few have investigated changes in neural activity on circadian timescales (Matzen et al. 2012; Talathi et al. 2009). Our study suggests a specific mechanism for how hippocampal 24-h rhythms become altered; as discussed below, these altered rhythms may relate directly to the emergence of epileptic seizures after injury.

**Variability of data in preinjury time period of nonseizing animals.** It was noted that preinjury data for the nonseizing animals showed a significant amount of variability, as evidenced by the size of the error bar in Fig. 4F. This may have resulted in part from the fact that technical difficulties prevented the full 7 days of preinjury data from being collected for one of these nonseizing animals. The data from the animal in question are shown as an example in Fig. 4E. Although this animal still passed the zero-amplitude test ($P = 0.000096$), the paucity of data meant that the phase estimate was less reliable; specifically, the confidence interval of this animal’s $T_{\text{SPKmin}}$ was ±2.3 h (95% confidence interval), almost double the largest of the remaining 4 (3 seizing and 1 nonseizing) animals’ confidence intervals (±1.3 h). While the paucity of data was one factor contributing to the variability of the preinjury data, another contributing factor may be the presence of a circadian driver that entrains not to the day-night cycle but to another contributing factor may be the presence of a circadian driver that entrains not to the day-night cycle but to another contributing factor may be the presence of a circadian driver that entrains not to the day-night cycle but to another.

**Implications of altered circadian rhythms for emergence of epileptic seizures and cognitive impairment.** It is traditionally thought that processes such as neuron loss and subsequent circuit rewiring contribute to the emergence of seizures by creating excitatory-inhibitory imbalances (Briggs and Galanopoulou 2011; Dudek and Spitz 1997; El-Hassar et al. 2007; Sloviter 2005). It is possible that altered circadian regulation could facilitate the creation of such excitatory-inhibitory imbalances. Our modeling work showed that reduced septal circadian input can produce a phase misalignment in the 24-h rhythms of pyramidal cells and basket cells (Figs. 7, C and D); we propose that this creates an “optimal time window” for seizure occurrence. A description of this phenomenon, and how we hypothesize it might interact with other epileptogenic processes to trigger the emergence of seizures, is provided in Fig. 9. Specifically, the phase shift appears during the latency period resulting from structural damage associated with SE (i and ii, Fig. 9). Although the optimal time window exists during the latency period, cell death and other changes resulting from SE suppress firing such that excitation is still balanced by inhibition (iii, Fig. 9). Over time, epileptogenic processes restore mean firing activity to preseizure levels (dotted line, Fig. 9A), but they cannot restore the correct balance of circadian drive (iv, Fig. 9). Thus, even if the daily mean firing rate of excitatory and inhibitory cells does not exceed preseizure levels, the presence of the phase shift will still promote a temporary, yet potentially important, excitatory-inhibitory imbalance during the optimal time window (v, Fig. 9).

The presence of this optimal time window at noon may reflect the 24-h rhythm of epileptic seizures, which are observed to cluster in the afternoon in both rodent and human TLE (Hofstra and de Weerd 2009; Loddenkemper et al. 2011; Quigg et al. 2000). Previously it has been hypothesized that the 24-h rhythm of epileptic seizures results from passive entrainment to the 24-h rhythms of neuromodulators (Quigg 2000). However, the findings of a phase shift support the notion that the 24-h rhythm of seizures may be actively driven by abnormal circadian regulation that promotes periods of excitatory-inhibitory imbalance throughout the day.

An additional property of the mechanisms underlying 24-h seizure patterns can be discerned based on cross-species comparison. Specifically, although rodents and humans with TLE have peak seizure occurrence in the afternoon, they have opposing activity patterns (nocturnal vs. diurnal), thus indicating that daily rhythms of seizures are not driven directly by sleep or wake activity. Thus the underlying mechanism must be phase-conserved across these species. Twenty-four-hour rhythms of medial septum firing activity, to our knowledge, have not yet been quantified in humans; however, melatonin is known to be phase-conserved (Quigg 2000). Whether the underlying mechanism is passive entrainment, as has been previously proposed, or an active response to a phase shift, we predict that the phases of the oscillations involved should be conserved across species.

Circadian alteration may also contribute to epilepsy-associated symptoms. For example, circadian rhythms have been implicated in memory formation (Gerstner and Yin 2010), and there is evidence that processes such as LTP are regulated on a 24-h cycle (Chaudhury et al. 2005). Since hippocampal sharp waves are important for LTP (Buzsáki 1996; Selbach et al. 2004), it is possible that the phase misalignment of hippocam-
pal SPK activity reported here might be relevant for memory impairment in epilepsy.

Is the phase shift permanent? One assumption of our model is that the phase of circadian drive to the hippocampus is constant. However, an alternative possibility is that the phase shift associated with SE is a dynamical phenomenon, whereby a circadian oscillator gets transiently pushed out of alignment and later reentrains to its original phase. Such transient phase shifts in CBT rhythms have been reported after epileptic seizures (Quigg et al. 2001), and the distinction between transient and persistent circadian alteration has been discussed in detail (Quigg 2000). During our analysis, we examined the phase of 24-h rhythms exclusively near the ending of our data sets to test for the possibility that these rhythms may begin to reentrain. However, we instead found that the phase shift persisted throughout the final days of our recordings (see APPENDIX, Fig. A2). This suggests that the phase shift may be a permanent feature of SE.

Generality of computer model to alternative sources of circadian perturbation. While our MRI characterization focused specifically on changes in the medial septum, our computer model displayed a phase shift in the circadian rhythm of basket cell firing due to changes in the melatonin circadian input when septal input was held constant (Fig. 7E). Similarly, a phase shift could also be observed for changes in the strength of the pyramidal circadian input (not shown). Therefore, the mechanism for producing a phase shift by altering the relative balance of circadian drive appears general and independent of the nature of the specific circadian drivers involved. Our MRI structural characterization of the medial septum suggests that damage to the septum is one source for producing altered circadian drive. Damage to the septum is also supported by previous studies (Gorter et al. 2001, 2003). However, it is possible that other circadian drivers could also be modified so as to contribute to the phase shift. For example, changes in melatonin levels have been reported in epilepsy patients, although there is conflicting evidence as to whether melatonin is increased or decreased in the absence of seizures (Bazil et al. 2000; Hofstra and de Weerd 2009; Schapel et al. 1995). Likewise, damage to other nuclei under circadian influence, such as orexin-secreting cells in the hypothalamus (Peyron et al. 1998; Selbach et al. 2004), histamine-secreting cells in the tuberomammillary nuclei (Yanovsky and Haas 1998), or serotonin-producing cells of the raphe nuclei (Assaf and Miller 1978; Kubota et al. 2003), may also be relevant. Similarly, transmission of nonvisual light cues from the retina, which have been shown to elicit direct responses in the hippocampus and other limbic and subcortical regions (Vandewalle et al. 2009), may also be impaired so as to contribute to the phase shift. In general, it is possible that any circadian or diurnal input that meets the following three criteria could be relevant for influencing the SPK phase shift: 1) it has the ability to

Fig. 9. Schematic of changes during epileptogenesis and their proposed influence on epileptic seizures. A: functional changes in neural firing activity during epileptogenesis. Pyramidal (PYR) and basket cell (BC) firing rates are shown, with key events as numbered. Dotted line indicates the preinjury average firing rate for pyramidal cells. Solid lines serve as a guide to the eye for changes in the ratio of excitation to inhibition; reduced vertical distance between these lines indicates an increase in the excitation-to-inhibition ratio. Key events (i–v) during epileptogenesis are described below. B: structural changes in the hippocampus and surrounding network during epileptogenesis. Key events are as follows: i. Prior to injury, PYR and BC activity are balanced throughout the day. ii. Injury from SE attenuates circadian input to the hippocampus from regions such as the medial septum, creating an imbalance in circadian drive. iii. This produces a phase shift in the firing of basket cell activity. Additional damage caused by SE, such as hippocampal cell loss (B, ii), alters the average daily firing activity and sets epileptogenic processes in motion. iv. Epileptogenic processes, including further cell loss, sprouting, and other homeostatic mechanisms, drive the emergence of spontaneous seizures. v. These processes act in part to restore the daily average levels of excitatory and inhibitory activity (dotted line). However, they do not restore the correct balance of circadian input, and thus the phase shift persists. The circadian phase shift produces a time window during which the ratio of excitation to inhibition is increased relative to the preinjury period, as illustrated by the reduced distance between the solid lines in A. This provides an optimal time window for augmented seizure occurrence.

J Neurophysiol • doi:10.1152/jn.00911.2012 • www.jn.org
influence the rate of hippocampal SPKs; 2) shows evidence of 24-h rhythms; 3) experiences damage or alteration following SE. While the medial septum meets these criteria, other sources of input may be relevant as well.

It is also feasible that connectivity within the CA3 network might be altered during epileptogenesis. We investigated this possibility by changing network convergences while holding other parameters constant and found that this could produce a phase shift under certain circumstances. For example, one such condition was a reduction in the convergence of pyramidal cells on basket cells. As pyramidal cells reached peak firing during the day, loss of their input to basket cells allowed the underlying melatonin circadian drive to promote peak firing at night, thereby producing a phase shift. Thus loss of principal cell input to basket cells due to necrotic processes might also be a contributing factor to a phase shift. This investigation underscores the principle that many parameter changes can lead to a phase shift via the same general mechanism, namely, a change in the balance of circadian drive to a given cell type.

Relationship between SPK phase shift and hippocampal neural firing activity. An implicit assumption in our computer modeling work is that the phase shift in the circadian rhythm of SPKs is related to hippocampal neural firing activity. While the neural mechanisms of SPK initiation are not yet fully understood, recent studies have shown that SPK rates are influenced by neuron firing. In particular, an in vitro study showed that direct stimulation of individual perisomatic-targeting interneurons, but not other cell types, was sufficient to affect the probability of CA3 population bursts (Ellender et al. 2010). Interestingly, our computer model also predicted that a phase shift would emerge specifically in basket cells firing after loss of septal input (Fig. 7). Basket cells were most susceptible to phase shifting because they were strongly influenced by both the septal and the melatonin circadian inputs. For example, while O-LM cells were also driven by the septal input, they were less influenced by melatonin owing to their lack of recurrent GABAergic connectivity. For other values of circadian scaling coefficients (specifically increased \( C_{\text{med}} \) and \( C_{\text{septal}} \)), we observed that phase shifts could occur for O-LM and pyramidal cells as well. Therefore, it is possible that changes in the firing patterns of other neuron types or, alternatively, changes in network properties such as synchronization might also contribute to the circadian phase shift.

Emergence of interictal spikes as possible driver for circadian phase shift. To summarize our analysis of the SPK phase shift in relation to theta rhythms, we observed the following: 1) theta rhythms do not shift phase after injury and 2) SPK activity exclusively within the non-theta state exhibits a phase shift. While the second point discounts the possibility that CS may contribute to the phase shift by “leaking” into the theta state after injury, there is yet another mechanism by which CS could be responsible for the phase shift. Specifically, it is possible that the phase shift could simply be due to an increase in the overall frequency of IS following injury, provided that these IS occur at a different circadian phase than the SPWs. Unfortunately, this possibility cannot be confirmed or denied without direct measurement of SPWs and IS. However, for several reasons, we propose that both SPWs and IS together shift toward peaking during the day after injury. First, given the similarities in SPW and IS mechanisms of generation (Buzsáki 1986; Suzuki and Smith 1987), it is most likely that they are affected by circadian drivers in a similar manner and, therefore, exhibit similar circadian phases. Second, our analysis of EEG rhythms has shown that circadian modulation of EEG rhythm amplitude in the beta and low gamma frequency ranges (22–65 Hz) also exhibits a phase shift following injury (Stanley DA, Talathi SS, Ni X, Huang L, Lai YC, Ditto WL, Carney PR, unpublished observations). Therefore, this identification of a secondary EEG feature that also phase shifts suggests that processes other than simply the emergence of IS are involved.

In summary, we have shown that there emerges a phase shift in the 24-h rhythm of hippocampal neural activity very early in epileptogenesis. This phase shift emerges relative to circadian rhythms of both CBT and hippocampal theta, suggesting that the phase shift is not triggered by changes in the SCN or by changes in daily activity rhythms. Using a detailed biophysical model, we have shown how, in general, changes in the relative balance of circadian inputs can produce a phase shift. Specifically, MRI investigations show signs of structural change in the medial septum, suggesting that alteration in circadian input via the septum may contribute to the emergence of the SPK phase shift. This alteration in the circadian regulation of hippocampal neural activity may be important for contributing to the peak daily timing of epileptic seizures and to the emergence of epilepsy-related cognitive impairment.

APPENDIX

Sensitivity of results to theta epoch detection algorithm. During our analysis of theta activity, we sought to determine the sensitivity of our results to the choice of theta epoch detection ratio threshold. To accomplish this, we calculated the phase of daily rhythms of theta activity for theta-to-delta ratio threshold values ranging from 1.0 to 3.0 in increments of 1.0. Our results are summarized in Table A1. This analysis shows that acrophase estimates were stable for each of the three ratio thresholds examined.

Sensitivity of EEG results to choice of smoothing window. In this section, we discuss the sensitivity of our EEG results to the choice of smoothing window. We explored a variety of smoothing windows and found that the acrophase estimates were largely independent of the choice of window. The 6-h, 90%-overlapping window was chosen to follow the procedure described in the supplementary material of our previous work (Talathi et al. 2009). Figure A1 compares this to 2-h averages with a 15-min sliding window. The preinjury, latency, and spontaneously seizing acrophase values were 23.80, 14.37, and 16.18 h, respectively, for the 2-h averaging and 23.45, 14.47, and 16.22 h, respectively, for our original choice of 6-h averaging. This suggests that our results are largely insensitive to changes in the smoothing window.

<p>| Table A1. Summary of changes in ( T_{\text{vs},\text{min}} ) for latency and spontaneously seizing stages of epileptogenesis relative to preinjury stage |</p>
<table>
<thead>
<tr>
<th>Theta-to-Delta Ratio Threshold</th>
<th>( \Delta T_{\text{vs},\text{min}} ) h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>-0.43 (P = 0.81) 1.70 (P = 0.06)</td>
</tr>
<tr>
<td>2.0</td>
<td>-0.65 (P = 0.71) 1.15 (P = 0.31)</td>
</tr>
<tr>
<td>3.0</td>
<td>-0.55 (P = 0.59) 1.81 (P = 0.19)</td>
</tr>
</tbody>
</table>

Pre, preinjury stage; Post-L, latency; Post-SS, spontaneously seizing. P values reported are for paired t-tests (\( N = 3 \) rats).
Fig. A1. SPK acrophase estimate is not affected by choice of averaging window. A: SPK rate analysis (as in Fig. 4, A and B) is shown for a 6-h, 90%-overlapping smoothing window. Data are shown for Pre, Post-L, and Post-SS stages of epileptogenesis. Days are marked from the start of preinjury recording in i and from the day of SE in ii. Estimation of acrophase (as in Fig. 4C) is shown in iii. B: SPK rate analysis obtained with a 2-h, 15 min sliding window. Acrophase values estimated from cosine fits are 23.45, 14.47, and 16.22 h for the 6-h averaging (A, iii) and 23.80, 14.37, and 16.18 h for the 2-h averaging (B, iii), respectively.

Fig. A2. Analysis of persistence of 24-h rhythm phase shift. A: SPK rates during the final days of recording. All data are taken from the postinjury spontaneously seizing stage (Post-SS) of epileptogenesis, from the same rat as shown in Fig. 4, A–C. Days are marked from the day of SE. Data from the solid vertical line onward mark the final 10 days of recording (Final-10), which was used for phase analysis. B: phase analysis performed on the marked region of data. All 3 rats showed SPK rates peaking in the afternoon, as was observed in the analysis of the entire Post-SS and latency (Post-L) stages in Fig. 4. C: the time of minimum SPK activity during the final 10 days of recording was significantly different from the preinjury stage and was not substantially different from the Post-L and Post-SS period measurements made in Fig. 4D. Values are means ± SE. *P < 0.05 by t-test.
Analysis of persistence of 24-h rhythm phase shift. Here we report our investigation of the possibility that the phase shift might be a transient phenomenon and that the hippocampal 24-h rhythms might return to their preinjury phase over time. To test this, we examined data exclusively near the end of the recorded data sets. We observed that, even during the final 10 days of recordings, the phase was still shifted significantly relative to the preinjury period ($P = 0.0113$), with the 24-h modulation of spiking activity peaking in the afternoon (Fig. A2). We expected that if the phase shift were due to an oscillator causing them to peak at noon ($S_{\text{midnight}}$). This primarily affects BC and O-LM interneurons, their firing rate to peak at midnight ($S_{\text{max}}$).

Response of CA3 network model to individual circadian inputs. To investigate the dynamics of circadian modulation within the model, we ran simulations in which circadian inputs were applied individually. This reveals how the effects of each circadian input propagated throughout the network to affect neuron firing rates. Figure A3 shows the effects of individually applying the CA3 pyramidal cell, medial septum, and melatonin circadian inputs. It was evident that individual circadian inputs had mostly intuitive effects; for example, the CA3 pyramidal circadian input, which promoted CA3 pyramidal cells to fire during the day, propagated through the network to produce maximum basket and O-LM firing during the day. Basket cells would in turn influence MSG cells to reduce firing during the day; however, the strength of this modulation was quite attenuated.

ACKNOWLEDGMENTS

We are grateful to T. Kiss for sharing code for the hippocampus model, W. Tripplett for his work on the fiber tracking code, and J. Stanley for proofreading the manuscript. We also thank M. Spano and R. G. Shivakeshavan for assistance with accessing computational facilities.

REFERENCES


Leung IW. Hippocampal interictal spikes induced by kindling: relations to behavior and EEG. Behav Brain Res 31: 75–84, 1988.


