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Circadian control of neural excitability in an animal model of temporal lobe epilepsy

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ABSTRACT

We provide experimental evidence for the emerging imbalance in the firing activity of two distinct classes (type 1 and type 2) of population spikes recorded from the hippocampal area CA1 in an animal model of temporal lobe epilepsy. We show that during the latent period of epileptogenesis following status epilepticus inducing brain injury, there is a sustained increase in the firing rate of type 1 population spikes (PS1) with a concurrent decrease in the firing rate of type 2 population spikes (PS2). Both PS1 and PS2 firing rates are observed to follow a circadian rhythm and are in-phase in control rats. Following brain injury there is an abrupt phase shift in the circadian activity of the PS firing rates. We hypothesize that this abrupt phase shift is the underlying cause for the emergence of imbalance in the firing activity of the two PS. We test our hypothesis in the framework of a simple two-dimensional Wilson–Cowan model that describes the interaction between firing activities of populations of excitatory and inhibitory neurons.
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"Balanced" networks in the brain have been proposed to account for a large variety of observations of cortical activity, including the representation of sensory information, decision-making and sleep and motor control [7]. A loss of balance in the neuronal network activity has been associated with the emergence of a number of neurological diseases including Parkinson's [15], Autism [18], Schizophrenia [22], and Tourette's syndrome [20]. Epilepsy, a neurological disorder of the brain in which patients suffer from recurrent seizures, is associated with an imbalance in the activity of excitatory and inhibitory populations of neurons in the brain, in favor of the former, leading to an abnormal hyper-synchronous state of the brain [4]. A number of *in vitro* studies have demonstrated the mechanism of this hyperexcitability at the synaptic level [8,11]. However, the functional implication of these synaptic changes leading to the progression of the brain to an epileptic state following brain injury in an *in vivo* system is still unknown.

Here we investigate the temporal dynamics of firing rates of high amplitude short time duration (100–200 ms) spatially localized patterns of spontaneous electrical activity referred to as *population spikes* (PS), recorded from the hippocampal CA1 area in an animal model of temporal lobe epilepsy. The PS are the macroscopic physiological features representing the integrated synaptic activity in the extracellular space generated by synchronous firing of populations of neurons in the brain [3,5]. Depending on the shape profile two distinct classes of PS were identified in neural recordings from the hippocampal CA1 area, labeled as type 1 PS (PS1) with a large negative excursion in the measured electrical activity and type 2 PS (PS2) with a large positive excursion in the measure electrical activity.

We observe that the firing rates of the two PS (defined as the number of spontaneous PS events observed per unit of time) exhibit circadian-like 24 h periodicity and are locked in-phase in control rats. However, during the latent period, defined as the time period following brain injury until the time of generation of first spontaneous epileptic seizures, while the firing rates of these PS are circadian, they are now locked in anti-phase. This phase shift is abrupt occurring within a few days post-brain injury and persists throughout the latent period. During the latent period we also observe an evolving imbalance in the firing rate of the two PS (quan-

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tified through an estimate of the drift in the baseline firing rate), such that there is a sustained increase in the firing rate of PS1 with a concurrent sustained decrease in the firing rate of PS2. We theorize that *this evolving imbalance may be implicated in the generation of the first spontaneous epileptic seizure following electrically induced status epilepticus*.

Based on these experimental findings, we hypothesize that the strength of the interactions between the populations of neurons in the hippocampus is dependent on their phase relative to the daily circadian cycle. Brain injury abruptly disturbs the circadian phase, which in turn triggers homeostatic mechanisms [9] producing changes in the interaction strength between the populations of hippocampal neurons which in turn modulates their firing activity. We refer to this as the "circadian-control" (CC) hypothesis. We suggest that this may underlie the cause for the emerging imbalance in the firing activity of the PS in the hippocampal CA1 area.

Our experiment used adult male Sprague Dawley rats ($n = 9$) of age 63 days and weighing between 200 and 265 g, which were implanted with 16 microwire recording electrodes (microelectrodes) bilaterally into the CA1 and the dentate gyrus regions of the hippocampus. In addition, a bipolar, twisted Teflon-coated stainless steel electrode was implanted into the right ventral hippocampus for the induction of brain injury [16]. The experimental details are given in the methods section of the supplementary material accompanying this manuscript. After 1 week of baseline recordings at a sampling rate of 12 kHz, rats ($n = 7$) were electrically stimulated for 30 min until sustained behavioral and electrographic seizures were observed. After the rats stopped seizing they entered a seizure-free latent period. Subsequently, rats were housed in a controlled environment with 24 h symmetric day–night cycle and monitored with continuous video and extracellular brain electrical activity recordings. Videos were screened daily for spontaneous seizures. At the end of the recording session, the rats were sacrificed and the intact brains were excised. The isolated intact brains were imaged with high-field magnetic resonance microscopy to confirm the location of the electrode placement within the CA1 region of the hippocampus [19,21]. In total 7 (E1–E7) rats were electrically stimulated into status epilepticus. A total of 3 rats (E1–E3) entered the chronic phase of epileptic seizures, following an epileptogenic phase with a minimum of a Racine grade 3 first spontaneous seizure. Data presented in this work is primarily derived from these 3 epileptogenic rats. Table 1 in the supplementary methods section summarizes the mean duration of epileptogenic phase and the Racine seizure grade for all the electrically stimulated rats. The rats (E4–E7) provided us with additional data-points to validate epileptogenic circadian modulation in firing activity of PS events following status epilepticus.

In Fig. 1A, we show a representative example of the extracellular activity recorded from the hippocampal CA1 area of an epileptogenic rat during the latent period. Overlaid on the trace, in squares and circles, we show the PS1 and PS2 events, respectively. In Fig. 1B and C, we show the mean shape profile of the PS1 activity and the mean shape profile of the PS2 activity detected from the same rat over a latent time period of 12 days of recordings using a modification of a well-known spike clustering algorithm [12].

The time evolution of the normalized firing rates of PS1 and PS2 from an age-matched control rat (C1) and during the latent period in an epileptogenic rat (E2) is shown in Fig. 2A–D. Key points worth mentioning from Fig. 2 are: (1) there exists a circadian-like modulation in the firing rate of the PS1 and PS2 activity both during the control and the latent time periods; (2) there is no observed drift in the firing rate of the PS1 and PS2 activity in the data obtained from control rats (Fig. 2A and C); (3) during the latent period there is a marked upward drift in the firing rate of PS1 and a corresponding marked downward drift in the firing rate of the PS2 (Fig. 2B and D); (4) the circadian-like modulation of firing rates of PS1 and PS2

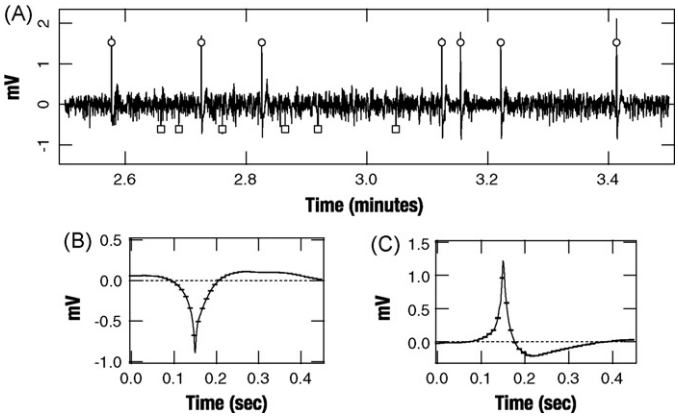

Fig. 1. (A) Sample 1 min trace of extracellularly recorded brain electrical activity from the hippocampal CA1 area. Overlayed on the trace are the times of occurrences of spontaneous population spikes, squares representing the type 1 population spikes (PS1) and circles representing the type 2 population spikes (PS2), (B) mean shape profile of PS1 and (C) mean shape profile of PS2.

are locked in-phase during the control period (Fig. 2E), while during the latent period the two PS oscillate anti-phase with respect to each other (Fig. 2F), with a marked shift in the rhythmic activity of PS1; (5) the average number of PS1 events per hour recorded during the latent period in the three epileptogenic rats are significantly greater ($p \approx 0.0026$; two-sample t-test) than that recorded during the control period, while the average number of PS2 events per hour are less ($p \approx 0.058$; two-sample t-test) during the latent period as compared to the pre-status epilepticus control period in these rat (Fig. 2G and H).

In Fig. 3, we summarize the results on the phase shift in the circadian-like firing activity of the two PS and the imbalance in their firing rates during the latent period from the PS data obtained from 3 epileptogenic (E1, E2, E3) and 2 controls (C1, C2). The imbalance in the firing rates is quantified by estimating the drift $D = \langle df/dt \rangle$ (f: firing rate) in the firing activity of both PS1 and PS2 through a least-squares fit of the drift in the baseline-firing rate to a straight line, $\Delta f = D\,\Delta t + c$. In Fig. 3A, we plot the mean value of D (with error bars representing the standard error corresponding to 95% confidence interval). From Fig. 3A, we see that, while the firing rates are in balance ($D \approx 0$) in controls, $D > 0$ during the latent period in epileptogenic rats ($p \approx 0.0044$, two-sample t-test). This implies an evolving imbalance in the firing activity of the two PS. The phase relationship between the circadian like firing activity of the PS1 and PS2 is quantified through a least squares-fit of the detrended-modulo 24 firing rate data (detrending implies the removal of the drift in the baseline of the circadian-like rhythm of firing rate) with a sinusoidal function $f(t) = a\sin(\omega t + b)$, with $\omega = 7.2722 \times 10^{-5}$ Hz. The phase is associated with the time T_X (X = PS1, PS2) of maximum value obtained by $f(t)$ and is given as: $\Phi_X = 2\pi T_X/24$. The mean value of phase for the two PS (with standard error corresponding to 95% confidence interval) is shown in Fig. 3B. The relative phase difference is quantified as $\Delta\Phi = |\Phi_{PS1} - \Phi_{PS2}|$. In-phase firing activity of the two PS is considered to occur when $\Delta\Phi \leq \pi/2$. We see that during the control period, the two PS events are phase-locked with a lag of around $\pi/4$ radians, however during the latent period, the phase lag increases to approximately $3\pi/4$ radians. The phase shift $\Delta\Phi$ in the relative phase for the epileptogenic rat is significantly greater than that for the control rat ($p \approx 7.3775 \times 10^{-5}$, two-sample t-test).

We have proposed a CC hypothesis, which suggests that the evolving imbalance in the PS1 and PS2 firing rates is the result of an abrupt phase-shift in their circadian activity. In order to study the implications of this hypothesis in the context of our experimental results as presented above, we consider a simple two-dimensional