The Hippocampus Participates in a Pharmacological Rat Model of Absence Seizures

Justin Arcaro^{a,b,c}, Jingyi Ma^b, Liangwei Chu^b, MinChing Kuo^b, Seyed M. Mirsattari^{a,c}, and L. Stan Leung^{a,b}

^aGraduate Program in Neuroscience, University of Western Ontario, London, Ontario, Canada

^bDepartment of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

^cDepartment of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada

SUMMARY

Objective—Using the gamma-butyrolactone (GBL) model of absence seizures in Long-Evans rats, this study investigated if 2.5–6 Hz paroxysmal discharges (PDs) induced by GBL were synchronized among the thalamocortical system and the hippocampus, and whether inactivation of the hippocampus affected PDs.

Methods—Local field potentials were recorded by chronically implanted depth electrodes in the neocortex (frontal, parietal, visual), ventrolateral thalamus and dorsal hippocampal CA1 area. In separate experiments, multiple unit recordings were made at the hippocampal CA1 pyramidal cell layer, or the mid-septotemporal hippocampus was inactivated by local infusion of GABA_A receptor agonist muscimol.

Results—As PDs developed following GBL injection, coherence of local field potentials at 2.5–6 Hz increased between the hippocampus and thalamus, and between the hippocampus and the neocortex. Hippocampal theta rhythm was disrupted when GBL induced immobility in the rats. The probability of hippocampal multiple unit firing significantly increased at 40 – 80 ms prior to the negative peak of thalamic PDs. Coherence between hippocampal multiple unit activity and thalamic field potentials at 2.5–6 Hz was significantly increased after GBL injection. Muscimol infusion to inactivate the mid-septotemporal hippocampus, as compared to saline infusion, significantly decreased the peak frequency of the PDs induced by GBL, decreased 30–120 Hz hippocampal gamma power, and hastened the transition of PDs to 1–2 Hz slow waves.

Significance—During GBL induced 2.5–6 Hz PDs, a hallmark of absence seizure, increased synchronization between the hippocampus and the thalamocortical network was indicated by frequency and temporal correlation analysis. These results suggest that the hippocampus was entrained by thalamocortical activity in the present model of absence seizures. Prolonged synchronization of the hippocampus may result in synaptic alterations that may explain the

cognitive and memory deficits in some patients with absence seizures and absence status epilepticus.

Keywords

absence seizure; thalamocortical network; gamma-butyrolactone (GBL); synchronization; local field potentials; hippocampus

INTRODUCTION

Absence epilepsy is a genetic epilepsy syndrome with generalized seizures that originate from local areas and rapidly engage bilaterally distributed networks (Berg et al., 2010). Typical absence seizures make up the primary seizure type in a number of absence epilepsy syndromes, including childhood absence epilepsy and juvenile absence epilepsy (ILAE, 1989), which differ mainly in age of onset. Seizures commonly manifest between the age of four years and early adolescence and are more prevalent in females. More common in adults are prolonged states of confusion with continuous and generalized EEG discharges known as absence status epilepticus (Agathonikou et al., 1998). The behavioral hallmark of absence seizures is a brief loss of awareness and staring spells with an abrupt onset and offset. There is an interruption of activity, lasting 2–20 s, and the individual normally has no recollection of the event (ILAE, 1989). The ictal electroencephalographic (EEG) pattern is symmetrical, bilaterally synchronous 3 Hz spike-and-wave discharges (SWDs). SWDs are associated with cortical neuronal firing during the EEG spike and neuronal silence during the wave (Steriade et al., 1994).

SWDs result from paroxysmal oscillations in the thalamocortical network, with possible involvement of the reticular projection systems of the brainstem and thalamus (Gloor, 1968; Meeren et al., 2005). In the feline penicillin model, paroxysmal oscillation appeared to start initially in the neocortex, and then entrained the thalamus (Avoli et al., 1983). Data from rat genetic models of absence seizures appear to favor a neocortical rather than a thalamic initiating site. In two genetic models of absence seizure - WAG/Rij and GAERS rats – SWD generation was found to start from the deep layers of the neocortex, specifically the somatosensory cortex (Meeren et al., 2002, 2005; Polack et al., 2007). Inactivation of the somatosensory cortex abolished SWDs in GAERS rats (Polack et al., 2009). Magnetoencephalogram and functional magnetic resonance imaging support a cortical rather than thalamic initiation of SWDs in patients with absence seizures (Bai et al., 2010; Westmijse et al., 2009).

A commonly used acute model that reflects clinical and pharmacological characteristics of human absence seizures is the gamma-hydroxybutyric acid (GHB) rat model (Snead, 1988; Snead et al., 1999; Venzi et al., 2015). When GHB is administered, rats experience an arrest of motor activity, staring and occasional twitching of the vibrissae and facial muscles, concomitant with 4 to 6 Hz paroxysmal discharges (PDs)¹ in the EEG. The administration of gamma-butyrolactone (GBL), a prodrug of GHB (Guidotti and Ballotti, 1970) has been

¹The term “paroxysmal discharges” was used instead of “spike-wave discharges” since GBL induced discharges may not have the conventional spike-wave components in the EEG (Venzi et al., 2015; this study).

shown to enhance reproducibility of the induced PDs. GHB acts on gamma-aminobutyric acid type B (GABA_B) receptors to generate experimental absence seizures (Liu et al., 1992; Snead et al., 1999; Crunelli and Leresche, 2002). GBL-induced PDs involve thalamocortical circuits, as suggested by electrophysiological recording (Banerjee et al., 1993) and functional imaging (Tenney et al., 2003). However, different thalamocortical structures appear to be involved in the PDs in the GBL versus the genetic model of absence seizures. The GBL-induced PDs were abolished by bilateral lesion of the thalamic mediodorsal or intralaminar nucleus (Banerjee and Snead, 1994), while lesion of the ventrobasal and reticular thalamic nuclei abolished the spontaneous SWDs in GAERS (Vergnes and Marescaux, 1992). In addition, GBL-induced PDs apparently involve mainly the superficial layers of the neocortex (Banerjee and Snead, 1994), while SWD generation in WAG/Rij and GAERS rats originate from the deep layers of the neocortex (Meeren et al., 2002; Polack et al., 2007).

Whether neural networks other than the thalamocortical system are involved during absence seizures have not been fully investigated, although a link between absence seizure mechanisms and limbic structures has been suggested (Onat et al., 2013). Specifically, we hypothesized that the hippocampus is entrained by thalamocortical PDs in the GBL model of absence seizures in the rat. The hippocampus is particularly interesting since its involvement may explain the different degrees of cognitive impairments and memory loss in typical or atypical absence seizures (Caplan et al., 2008; Onat et al., 2013; Jackson et al., 2013). Previous recordings in genetic absence rodent models did not find evidence of hippocampal involvement during SWDs (Vergnes et al., 1990; Inoue et al., 1993; Kandel et al., 1996). In the GBL model in rats, there was also no apparent hippocampal participation in PD generation (Banerjee et al., 1993), but synchronization of hippocampus with thalamocortical activity was inferred by signal analysis (Perez Velazquez et al., 2007). In the course of our study, we found evidence of entrainment of the hippocampal neural activity from PDs induced by GBL, and further asked the question whether the hippocampus was necessary for generating the PDs. We used reversible chemical inactivation of the hippocampus by muscimol, a GABA_A receptor agonist, to answer the latter question.

METHODS

1.1 Animals

Adult male Long-Evans rats (Charles River, Canada), weighing 250–400 grams, were kept on a 12:12 hour light-dark cycle, starting at 7:00 with food and water freely available. Experiments were conducted between 9:00 and 19:00 hour, in accordance with the guidelines established by the Canadian Council on Animal Care and approved by the local Animal Use Committee.

1.2 Surgery & Electrode Implantation

Rats were anesthetized with sodium pentobarbital and secured in a stereotaxic frame. In 10 rats intended for multi-site local field potential (LFP) recordings, electrodes (Teflon-coated stainless steel wires of 127 µm diameter) were targeted at the following coordinates relative to bregma (Paxinos and Watson, 2009), with lambda and bregma in a horizontal plane: right

frontal cortex (anterior 1.4, lateral (L) 2.0, ventral from skull surface (V) 1.5, all units in mm), right ventrolateral thalamic nucleus (posterior (P) 2.4, L 2.2, V 6.0), right visual cortex (P 7.0, L 3.0, V 1.5), left hippocampal CA1 region (P 3.2, L 2.2, V 3.0) and left parietal cortex (P 3.2, L 2.2, V 1.5) (Supplementary Fig. 1A). In 6 rats intended for hippocampal neuronal recording, Teflon-coated stainless steel wires (76 μ m diameter) were placed bilaterally in the CA1 pyramidal cell layer at a mid-septotemporal level (P 4.4, L 2.4, V 3.0; Supplementary Fig. 1B), using electrophysiological criteria (Leung, 1979), with additional electrodes placed in the ventrolateral thalamus and parietal cortex. In 2 rats, a silicon electrode array (Neuronexus, Ann Arbor, MI), of 16 electrodes separated by 50 μ m, was chronically implanted into the dorsal hippocampus (P4.0, L2.7). A jeweller's screw in the skull over the left cerebellum, or another in the left frontal skull, served as a recording ground.

Eight rats were implanted with 23-gauge stainless steel guide cannulae bilaterally into the hippocampus (Ma et al., 2002) at P4.6, L2.5, V3.0 (Supplementary Fig. 1B). The hippocampal CA1 area is known to receive afferents from the midline thalamus (Dolleman-Van der Weel et al., 1997). An electrode was glued to the anterior side of the guide cannula, and wire electrodes were also implanted into the ventrolateral thalamic nucleus bilaterally and the right frontal cortex (coordinates above).

All electrodes and cannulae were fixed by creating a head cap made of dental cement. Experiments did not commence until at least a week after surgery for recovery.

1.3 Local Field Potential Recordings

Rats were placed in a Plexiglas recording cage in the laboratory and habituated for 2–3 days before experiments, including adapting to being connected to a flexible recording cable. For multiple cortical site recordings, LFPs were amplified by a Grass Model 8–10 system and filtered between 0.3–70 Hz. Baseline LFPs were recorded for at least 40 min before GBL injection, which included the gross behavioral state of awake-immobility, which is operationally defined when the rat showed no gross head or body movements and held its head up against gravity. In some rats, baseline recording included slow-wave sleep, which was operationally defined when the rat assumed a sleep posture, and with high-amplitude slow (< 2 Hz) waves in the neocortical and hippocampal LFPs. An intraperitoneal (i.p.) injection of GBL (200 mg/kg) was then made and LFPs were recorded for 30–120 min following injection.

1.4 Hippocampal Neuronal Unit Recordings

Hippocampal and thalamic signals from the rat were amplified and filtered between 0.3 Hz and 10 kHz (first order bandpass filter) by a Grass 7P511 amplifier. The hippocampal unit activity signal was further amplified by 500–1000 times and highpass filtered at 250 Hz (second order filter) using Intronix signal conditioners. LFPs and unit signals were then fed into a Data Translation D303 analogue-to-digital converter and sampled and stored at 10 kHz by custom-made scripts written in SciWorks 7 (DataWave Technologies, Loveland, CO). Multiple unit activity from the hippocampus was used since this gave more reliable temporal correlation with LFP events, as compared to single pyramidal cells which fired at

low rates (Buzsáki et al., 1983). Once the rats habituated to the recording condition, baseline recordings were collected for 30 minutes, which always included periods (> 6 min) of awake-immobility. GBL (200 mg/kg i.p.) was then injected and LFPs and neuronal units were recorded for two hours. During unit acquisition, the detection threshold of a neuronal unit was set to at least twice the amplitude of the background, and the waveforms (0.6 ms before and 0.6 ms after the detected peak) of all detected units were stored during acquisition.

1.5 Muscimol inactivation of the hippocampus

In 8 rats, after recording baseline LFPs during immobility and walking, a 30-gauge inner cannula was inserted into the guide cannula, and 1 μ l of saline or muscimol (1 μ g/ μ l), was infused over 3 min into the hippocampus bilaterally. Immediately following infusion, GBL was injected at 200 mg/ml i.p., and LFPs (from hippocampal and thalamic electrodes bilaterally, and right frontal cortex) were recorded continuously for at least 30 min. Based on ~1 mm radius spread from the center of infusion (Martin, 1991, Ma et al., 2002), it was estimated that ~30% of the dorsal hippocampus, and little of the ventral hippocampus, was affected by bilateral injection of muscimol. Each rat was given both saline and muscimol infusion, in experiments separated by at least 7 days, randomly starting with saline or muscimol first.

1.6 Data Analysis

1.6.1 Spectral analysis of Local Field Potentials—Each segment was tapered (10% of segment at each end) by a cosine bell function, and the auto- and cross-power (coherence and phase spectra) were obtained by Fast Fourier Transform (Leung et al., 1982). The coherence is the squared coherence $C_{xy} = |P_{xy}(f)|^2 / (P_{xx}(f) * P_{yy}(f))$, where $P_{xx}(f)$ and $P_{yy}(f)$ are autopower spectra of temporal signal x and y respectively, and $P_{xy}(f)$ is the cross power spectrum. Auto and cross spectra of all the selected segments were averaged, and then smoothed by an elliptical function (across $2*nf+1$ points, where nf = one side band width). For multi-site LFP recordings, each segment had 1024 points sampled at 200 Hz (Leung et al., 1982), giving the spectra a frequency resolution of 0.19 Hz; averaging >6 segments with $nf=2$ smoothing gave > 60 degrees of freedom (df). For unit-LFP spectra, each segment had 8192 points sampled at 10 kHz, giving frequency resolution of 1.23 Hz; and >20 segments were averaged and smoothed by $nf=1$ to give >120 df. For LFPs in muscimol/saline infusion experiments, 4096 points (4.096 s) were sampled at 1 kHz, and resolution was 0.24 Hz, with $nf=2$ and >100 df.

The change of peak power with time at all recorded channels was analyzed by short (1-s) segment power spectral analysis, with a Matlab specgem subroutine. From the power spectra obtained from each time segment, a power maximum (irrespective of magnitude) in the 2.5–8 Hz frequency range was identified; if the latter maximum was not present, the peak frequency was assigned to that with the maximal absolute power in the 0–2.5 Hz range. The temporal change of peak frequency of one channel was compared to that of another channel, at a fixed time after GBL injection or at a fixed stage (bursting versus continuous PD stage). The temporal relation of the change of peak frequency across channels was estimated by the

cross-correlation coefficient of the peak frequency values calculated for different pairs of recorded channels.

For a group of rats, the logarithmic power and the coherence spectra were averaged across rats, and compared between different conditions. At each digital frequency, a paired Wilcoxon test was used to assess if the power/coherence was different between two conditions (baseline, bursting and continuous stages of PD) with probability $P < 0.05$. Spectra between the two conditions were considered statistically different from each other within a frequency range where 5 contiguous digital frequencies (~1 Hz range) showed $P < 0.05$ difference individually.

Nonparametric Wilcoxon, Kruskal-Wallis chi square, and Dunns Multiple Comparison post-tests were conducted to determine statistical significance. Two-way (group x time) ANOVA was used for analyzing the LFP frequency and power following GBL injection, after infusion of saline or muscimol into the hippocampus.

1.6.2 Hippocampal Neuronal Unit Recordings—Multiple unit activity was selected mainly by a voltage discriminator set at >2 times the background noise; further selection could be made by applying cluster separation using other parameters of the unit waveforms in a DataWave Autosort routine. For each rat, files with 3-min data were chosen from the baseline, or at different times after GBL injection, for analysis by scripts written in SciWorks and Matlab. Two main procedures were used to identify the temporal relationship between hippocampal unit firing and thalamic or other LFPs: (i) The negative peak of the thalamic PD was used as an event to trigger peri-event time histograms (PETHs) of unit firings, and for averaging the thalamic LFP, from 500 ms before to 500 ms after the event. The statistical significance of the number of units in a 10-ms bin of the PETH was estimated by binomial theorem. It was assumed that each bin per trigger (sweep) can be either 1 (fired) or 0 (not fired), and the mean probability of firing is the number of above-threshold spikes in 3 min, divided by 18,000 bins ($180 \text{ s} \times 100 \text{ 10-ms bins/s}$). Unit firing at -100 to 100 ms of the trigger was considered significant if the Bonferroni-adjusted probability was $P < 0.01$. An additional Matlab script was also available for only selecting segments (of 0.819 s duration) for PETH and triggered LFP analysis only if the segment showed a main LFP frequency within a certain range. (ii) Cross spectral (coherence and phase) analysis (implemented with Matlab programs) was applied to the unit activity and a (thalamic or hippocampal) LFP (Buzsáki et al., 1983; Leung and Buzsáki, 1983). Each detected unit was considered a pulse, and converted to analog data (Leung and Buzsáki, 1983); the unit analog activity is the sum activity of all the converted pulses within the time segment. Frequency-selected segments were used, after analyzing the frequency of the maximal power in each segment of 8192 points (0.819 s sampled at 10 kHz); averages were made for groups with a discrete peak frequency of 2.47, 3.70, 4.93, 6.17, 7.40, and 8.63 Hz, i.e., the range of frequency of PDs and high-voltage spindles. Randomly shuffled surrogates were used to estimate the statistical significance of the experimental coherence value. The same data that resulted in the experimental cross spectrum were used, except each sweep of LFP was now paired randomly with another sweep of unit activity that was not recorded simultaneously, and the same number of sweeps was used to construct an average spectrum. At least 200 surrogates

were used, to give a probability of $P < 0.005$. Values are presented as mean $\pm$ standard deviation (SD).

1.7 Histology

Following all experiments, the rats were anesthetized with pentobarbital (80 mg/kg i.p.). A small lesion was made by passing a current ($0.5 \text{ mA} \times 3 \text{ s}$) through the electrodes of interest. The rat was transcardially perfused using 4% formaldehyde, and the brain was then cut into 60 μm coronal sections by a freezing microtome. Brain sections were stained with thionin to identify the position of electrodes and cannulae (Supplementary Fig. 1).

RESULTS

2.1 Change of Local Field Potentials during GBL-induced seizures

Generalized seizures induced by GBL were defined by LFPs and behavior of the rat. During awake immobility in the rat, before GBL injection, low-voltage desynchronized activity was typically observed in the neocortex, accompanied by irregular slow activity in the hippocampus (Fig. 1B, baseline left part). In many rats, occasional, high-amplitude 6–9 Hz activity called high-voltage spindles (HVS) could appear in the neocortex and thalamus during baseline immobility (Fig. 1A, B; Vanderwolf, 1975; Semba et al., 1980; Buzsáki, 1991; Shaw, 2004). An episode of HVS consisted of a burst of 6–9 Hz spike-and-wave discharges that waxed and waned over 1–4 s duration (Fig. 1C). Even when obvious HVSs were excluded, power spectral analysis of LFPs during baseline immobility revealed a small power peak at $\sim 6.5 \text{ Hz}$ at most electrodes, including frontal cortex, ventrolateral thalamus and hippocampus (Fig. 2A).

About 4 minutes after GBL injection, the rats showed abnormal behaviors including chewing, intermittent staring spells, facial and vibrissae twitching, as 4–6 Hz PDs appeared in bursts at all electrodes (Fig. 1A, B; Supplementary Fig. 2). The PDs contained bursts of initial spike-wave discharges, followed by more oscillatory waves (Fig. 1A, B). This has been termed the bursting stage of SWDs (Snead, 1988; Banerjee et al., 1993), here called the bursting PD stage, to avoid labelling all discharges as spike-waves. The PDs appeared at similar times at neocortical (frontal, parietal, visual), thalamic and hippocampal electrodes bilaterally (Fig. 1, Supplementary Fig. 2). An episode of bursting PD at the thalamus and neocortex also waxed and waned like HVS, but for a longer period of time and at a low frequency (Fig. 1C). Two to 5 minutes after the appearance of the PD bursts, the LFPs remained rhythmic at 3–5 Hz, but showed relatively uniform amplitude/power across time (Fig. 1; Supplementary Fig. 2A). This has been called the continuous stage of PDs (Snead, 1988; Banerjee et al., 1993). The ‘continuous’ PDs appeared as 3–5 Hz activity that may not be completely constant in a time-frequency spectral plot, and with relatively low sharp components (Fig. 1A; Supplementary Fig. 2A). During the continuous stage, the rats became immobile, but eyes were open and apparently with a fixed stare. While the rat would not spontaneously initiate movements, it could respond to auditory clicks with flicks of the pinna, or to moderate, sudden touch with a slight flinch. In this study, the continuous PD stage was also operationally defined when the LFP peak frequencies (for most electrodes) stayed within the 2.5–6 Hz range. The duration of continuous PDs varied from 10 to 50 min

among rats, mainly because the peak LFP frequency in some rats fell below 2.5 Hz. In other rats, peak LFP frequency stayed within the 2.5–6 Hz range at all times after GBL injection. Irrespective of the lowest LFP peak frequency after GBL injection, the initial sequence of bursting and continuous PD stages was observed. The behavioral sedation ended at ~50 min after GBL injection, when spontaneous movements again appeared, usually starting with eating, chewing and head movements, and continued with normal walking 10–20 min later. LFPs when walking resumed resembled those of baseline.

The PDs were subjected to spectral analysis, and the group average power spectra are illustrated for frontal cortex, thalamus and hippocampal LFPs (Fig. 2). During the bursting PD stage, there was a large increase in power at ~5 Hz, and a general power increase in the 0–30 Hz range, as compared to baseline awake-immobility (Fig. 2A); a statistically significant increase in power at a specific frequency, as compared to baseline, was indicated by a circle on the PD spectrum (Fig. 2A). Group average coherence at 4–6 Hz, for the hippocampus-frontal cortex and ventrolateral thalamus-hippocampus electrode pairs, but not for the frontal cortex-thalamus pair, increased significantly during the bursting PD stage as compared to baseline (Fig. 2C). Among the 10 pairs of electrodes in 8 rats, statistically significant increase of the peak coherence in the 2–9 Hz range was found in 5 pairs (all 5 pairs, Kruskal-Wallis chi square = 3, $P < 0.05$), and 4 of the 5 pairs involved the hippocampus (Table 1). Other than the pairs illustrated in Fig. 2C, statistically significant pairs were hippocampus-parietal, hippocampus-visual cortex and ventrolateral thalamus-parietal cortex.

During the continuous PD stage, the peak PD frequency decreased to 2.5–4 Hz (Fig. 1A,B, Supplementary Fig. 2A). Comparison between group power spectra during the continuous PD stage showed a significant downward shift in peak PD frequency as compared to the bursting PD stage (Fig. 2B), as well as a decrease in 10–30 Hz power at all electrodes (Fig. 2B). In the group average, the 2.5–4 Hz power peak was small, and tended to merge with a low frequency peak at 1–2 Hz (Fig. 2B). A downward shift in peak frequency from bursting to continuous PD stage was also evident in the group-averaged coherence spectra (Fig. 2C), but this was not statistically significant for the group (not shown in Fig. 2C; Table 1).

The peak frequency of the PDs was similar across all electrodes, and this decrease with time after GBL injection, from ~5 Hz during the bursting PD stage and decreasing during the continuous PD stage. When the time sequences of peak LFP frequency at different electrodes (hippocampus, ventrolateral thalamus, parietal, frontal, and visual cortex) were evaluated in fixed time segments, the cross correlation between peak frequencies of two channels was low (correlation coefficients of 0.13 to 0.31) for 1-s segments, but high for long segments of 30–82 s duration (correlation coefficients of 0.68 to 0.82). The average peak frequency at one electrode was not significantly different from that at another electrode when a segment duration of 30–82 s was used. This suggests that the peak frequency of PD was similar across channels but only at a longer (>30 s) than a short (1 s) time scale.

2.2 Hippocampal Neuronal Firing increased around the PD peaks

2.2.1 Event-triggered unit and field activity—The increase in coherence of LFPs suggests an increase in synchronization between the hippocampal and thalamocortical activities that occurred during PDs. However, laminar profiles of the PDs did not reveal clear

cellular generators in the hippocampus (Supplementary Fig. 3). The relation between hippocampal and thalamocortical activities was further studied by recording multiple unit activity in the hippocampal CA1 cell layer (Fig. 3A).

The average rate of multiple unit firing of hippocampal neurons generally decreased from baseline after GBL injection. During baseline, triggering from the thalamic HVSs did not yield statistically significant firing in the hippocampal unit PETH in 4 of 6 rats, as shown in a representative example (Fig. 3C); in 2 of 6 rats, PETHs (3 min recording) acquired during baseline immobility (with HVS) occasionally yielded statistical significant firing. During PD activity induced by GBL, PETHs consistently showed statistically significant hippocampal unit firing within 100 ms before the thalamic negative LFP peak, as illustrated by a representative rat (Fig. 3B). The same result was found for unit activity from a group of 6 different hippocampal electrodes in 5 rats, for a period 5–20 min after GBL injection. The mean hippocampal firing of the group ($N=6$ electrodes) peaked at 40–80 ms before the negative PD peak at the thalamus at time zero (Fig. 3D), and the firing peak was significantly different from zero ($P<0.05$). When sweeps of selected LFP peak frequency were selected, PETH and averaged LFPs triggered by the negative PD peak tended to show oscillatory multiple peaks (Supplementary Fig. 4B), while selected non-oscillatory PETHs showed only a single peak followed by a decrease in firing (Supplementary Fig. 4A).

2.2.2 Spectral analysis of oscillatory unit activity—Since rhythmic activities are favorably analyzed in the frequency domain, cross spectral analysis was used to assess the coherence and phase relation between hippocampal unit activity and rhythmic field potentials (PDs). Hippocampal unit activity was thresholded (tUA), and subjected to an anti-aliasing temporal filter (fUA in Fig. 4A; Methods 1.6.2), and cross spectral analysis was done with segments of the thalamic LFP of a selected peak frequency. When segments with HVSs (6–9 Hz peak power) in the thalamic LFPs during baseline (Fig. 4A1) were selected, the thalamic LFP power peaked at 7.4 Hz (Fig. 4B1), the hippocampal unit power peaked at 4.9 Hz (Fig. 4B2), while the LFP-unit coherence showed a small peak of 0.07 at 4.9 Hz, which was statistically different from zero (*, $P<0.005$; Fig. 4B3). At 9 min after GBL, the thalamic LFPs showed a power peak at 4.9 Hz (Fig. 4B1), with no clear unit power peak (Fig. 4B2) but a highly significant LFP-unit coherence of 0.14 (**, $P<0.0001$; Fig. 4B3). At 13 min after GBL, as compared to 9 min post-GBL, the thalamic LFPs peak frequency decreased to 3.7 Hz (Fig. 4C1), together with a decrease in both unit power and coherence, but the LFP-unit coherence remained significant (*, $P<0.005$; Fig. 4C3).

In a group of 6 confirmed unit recording sites in the hippocampus of 5 rats, PDs of frequency of 3.7–4.7 Hz gave average unit-thalamic LFP coherence of 0.24 ($SD=0.17$, $n=6$) at 8–13 min after GBL injection. This compares with unit-thalamic LFP coherence peak during baseline (selected for HVS) of 0.077 ($SD=0.07$), at frequency 6.99 Hz ($SD=1.84$ Hz, $n=6$). The unit-LFP coherence in the 2.5–9 Hz range increased from baseline to 8–13 min after GBL ($P<0.05$, paired Wilcoxon test). Based on unit-LFP spectral analysis, the positive peak of unit firing, calculated using individual phase shifts, occurred at 71 ms ($SD=58$ ms, $n=6$) before the negative peak of the thalamic LFP, a value consistent with the PETH analysis above.

2.3 PDs following saline or muscimol infusion into the hippocampus

Reversible inactivation of the hippocampus was used to study whether the hippocampus was essential for the generation of PDs. GABA_A receptor agonist muscimol, or saline, was infused into the mid-hippocampal CA1, near the subiculum (Supplementary Fig. 1B), and then 200 mg/kg i.p. GBL was injected. In the representative rat illustrated, hippocampal and thalamic PDs appeared after GBL injection, after either muscimol or saline pre-infusion in the hippocampus (Fig. 5A). However, after muscimol as compared to saline pre-infusion, PDs appeared to have lower frequency at a fixed time, and the peak frequency of PDs dropped quickly below 2.5 Hz (Fig. 5A). In a group of 6 rats with confirmed bilateral cannula locations in the hippocampus, the average peak LFP frequency at a hippocampal electrode was plotted at different times before and after GBL injection (Fig. 5B). During baseline, 7–8 Hz theta rhythm was recorded during walking, and 5–6 Hz peaks during immobility. After muscimol/ saline infusion followed by GBL injection, the mean peak frequency of the hippocampal LFPs (evaluated for one full minute) decayed gradually with time. The peak frequency was significantly lower with muscimol than saline pre-infusion [two-way block repeated measures ANOVA infusion effect $F(1,5) = 7.18$, $P < 0.05$; time effect $F(13,65) = 17.62$, $P < 0.0001$]. A similar effect of muscimol and saline on the decrease in LFP peak frequency with time was found at the frontal and thalamic electrode. Peak power at the hippocampal or the thalamic electrode was not significantly different (two-way repeated measures ANOVA, $P > 0.05$) between groups with saline and muscimol infusion into the hippocampus. Hippocampal and thalamic LFP gamma power decreased from 5 – 20 min after GBL injection, and hippocampal gamma power decrease was significantly larger after muscimol than saline pre-infusion (Supplementary Fig. 5).

DISCUSSION

Hippocampal involvement in GBL-induced PDs was investigated by analysis of the relation between hippocampal LFPs and hippocampal unit activity with other thalamocortical LFPs, in both time and frequency domains. Following GBL injection, Long-Evans rats showed PDs of 2.5 – 6 Hz, accompanied by symptoms of behavioral arrest and facial/vibrissal twitching, which are the characteristics of absence seizure rat models (Snead et al., 1999). Cross spectral analysis of LFPs indicated that the coherence of the hippocampal LFPs with other thalamocortical LFPs increased at the PD frequency. Analysis of the multiple unit activity of the hippocampus showed that units fired 40–80 ms before the negative peak of the thalamic PD, as confirmed by PETHs and cross spectral analysis of unit to thalamic LFP.

Five of the ten analyzed pairs of LFPs demonstrated a significant increase in coherence following GBL injection, and all pairs except one included the hippocampus. During bursting PDs, the hippocampal CA1 area exhibited enhanced LFP coherence with the thalamus and the parietal, frontal, and visual cortex. The increase in coherence of field potentials between two distant brain areas suggests functional connections between the two regions, either directly or through another common area (Leung, 2010).

The increase in coherence between hippocampal and thalamocortical LFPs after GBL occurred without a clear spatial maximum of the field potential amplitude in the hippocampus (Supplementary Fig. 3). The lack of a spatial current generator of SWDs in the

hippocampus has been reported previously (Vergnes et al., 1990; Kandel et al., 1996; Banerjee et al., 1993), and current source density analysis yielded no clear current sources or sinks in the hippocampus of WAG/Rij rats during SWDs of 9–11 Hz (Kandel et al., 1996). It is possible that spatially extensive thalamocortical structures may contribute, by volume conduction, to the amplitude of PDs recorded in the hippocampus, thus resulting in an increase in coherence involving the hippocampus. In order to exclude volume conduction, we directly recorded from neurons in the hippocampus.

Hippocampal multiple unit activity showed statistically significant correlation with the PDs recorded in the thalamus. PETHs showed that the hippocampal multiple unit activity peaked at 40 – 80 ms preceding the negative peak of the thalamic PDs. In addition, the coherence between hippocampal unit activity and thalamic LFPs increased from a low mean value of 0.077 during baseline HVS activity to a highly significant value of 0.24, with the positive peak of unit activity estimated to precede the negative thalamic LFP peak by ~70 ms. Previous recordings of hippocampal unit activity in WAG/Rij rats showed no oscillatory PETH triggered by HVSs of 9–11 Hz (Vergnes et al., 1990; Kandel et al., 1996), similar to our PETHs of hippocampal CA1 units triggered by baseline HVSs (Fig. 3C). The lack of significant unit-LFP temporal correlation may partly result from triggering from a thalamic LFP with variable peak amplitude and frequency. When spectral analysis was performed, a small but statistically significant unit-LFP coherence was found in the 4–8 Hz range of the HVSs (Fig. 4B). The present results differed from previous studies (Vergnes et al., 1990; Kandel et al., 1996) in using frequency-selected sweeps for spectral analysis, and in recording from mid-hippocampal CA1 neurons near the subiculum, where midline thalamic projection was expected to be maximal.

HVS activity in the neocortex of Long-Evan rats has long been known to be associated with vibrissal and facial muscle movements (Vanderwolf, 1975; Semba et al., 1980; Shaw, 2004). More recent studies indicated that HVS involved thalamocortical structures (Buzsáki, 1991; Kandel and Buzsáki, 1997), and was suppressed by anti-absence anticonvulsants, such as ethosuximide and valproate (Shaw, 2007), similar to SWDs in spontaneous genetic models of absence seizures (Coenen et al., 1992), or PDs induced by GBL (Snead et al., 1999). Despite a difference in frequency (PDs: 2.5–6 Hz; HVSs: 6–9 Hz; Fig. 1A), the waveform and development of a HVS and a bursting PD appear to be similar, e.g., in showing more “spiky” neocortical and thalamic LFPs during onset, and more wave-like oscillations at the end (Fig. 1C). While the frequency-selected coherence of hippocampal unit to thalamic LFP was higher for GBL-induced PDs than HVSs, other properties (response to drugs, spike-wave morphology, relation to vibrissal movements) appear to be similar between PDs and HVSs. However, the detailed thalamocortical generating mechanisms of GBL-induced PDs have not been reported.

The time-locked firing of hippocampal units shortly before the thalamic PDs provides evidence that hippocampal firing was synchronized with the thalamocortical PDs. How the hippocampal units fire 40–80 ms in advance to the thalamic negative PD is not known. It is known that thalamic inputs can excite hippocampus (CA1 and subiculum) neurons (Dolleman-Van der Weel et al., 1997; Bertram and Zhang, 1999; Vertes et al., 2007). Intrinsic resonance of hippocampal pyramidal cells at 4–10 Hz (Leung and Yu, 1998) or a

decrease in inhibition (Perez Velazquez et al., 2007) may enhance the response to rhythmic excitation. The hippocampus, in turn, can activate specific thalamic and cortical areas, possibly indirectly through the prefrontal and cingulate cortex, midline thalamus or mesopontine brainstem (Carr and Sesack, 1996; Vertes et al., 2007; Leung et al., 2014).

PDs were induced by GBL following muscimol inactivation of the hippocampus, suggesting that the hippocampus was not essential in PD generation by the thalamocortical system. The present muscimol infusion was estimated to affect <30% of the dorsal hippocampus, centered at CA1 near the subiculum. However, at a fixed time after GBL injection, the peak frequency of PDs was lower after muscimol as compared to saline pre-infusion into the hippocampus, suggesting that neural activity of the hippocampus contributes to general maintenance of PDs of a specific frequency. We reported a similar effect that hippocampal inactivation enhanced the loss of righting response, decreased gamma power, and increased <2 Hz power in the hippocampal and neocortical LFPs in response to a general anesthetic (Ma et al., 2002). GBL is known to have hypnotic effects (Guidotti and Ballotti, 1970), also suggested by its effect in decreasing PD peak frequency, increasing < 2-Hz power and decreasing gamma power in the LFPs (Supplementary Fig. 5). The hypnotic effects of GBL were enhanced by muscimol inactivation of the hippocampus, and they may shorten the period of 2.5–6 Hz PDs. The hippocampal inactivation effect may be mediated by a limbic network that involves basal ganglia and brainstem (Leung et al., 2014), perhaps overlapping with the brainstem-reticular network for PD generation as proposed by Gloor (1968).

Recent data suggest subtle changes in the hippocampus and limbic areas in models of absence seizures. Metabolic activity measured by glucose utilization was found to be significantly increased in the hippocampus of GAERS (Nehlig et al., 1998). WAG/Rij rats were reported to have a low threshold for the spread of epileptic activity in the neocortex and limbic structures (Tolmacheva et al., 2004), and injection of antiepileptic drugs into the hippocampus reduced the occurrence of SWDs (Tolmacheva and van Luijckelaar, 2007). Adding to the previous result of phase synchronization between hippocampal and thalamocortical activity during GBL-induced PDs (Perez Velazquez et al., 2007), our present findings provide direct evidence that the hippocampus was activated and entrained during PDs induced by GBL.

Atypical absence seizures, as compared to typical ones, have slower (< 2.5 Hz) SWDs, are more frequently associated with motor symptoms and severe cognitive and neurological impairments (Markand, 2003) that suggest hippocampal and limbic dysfunction (Onat et al., 2013). The degree of hippocampal and limbic involvement during SWDs may depend on absence seizure types, such as atypical absence or absence status epilepticus (Agathonikou et al., 1998). Hippocampal involvement may be a factor that explain the variability of cognitive and memory deficits among patients with absence seizures (Jackson et al., 2013).

In this study, we showed that GBL-induced PDs have behavioral and electrophysiological characteristics that are associated with absence seizures. Behavioral symptoms such as muscle twitching were most prominent during the bursting stage of PDs (see also Venzi et al., 2015), and motor symptoms commonly accompanied atypical absence seizures. The continuous stage of GBL-induced PDs was mainly associated with immobility and

diminished response to sensory stimuli; the prolonged duration of PDs, often mixed with low-frequency (< 2-Hz) activity, suggested absence status epilepticus. The GBL-model of absence seizures may not reproduce all the symptoms of either typical or atypical human absence seizure, but its validity lies in elucidating mechanisms and therapeutics. As shown by the present results, the GBL-induced model showed synchronization of the PDs between the thalamocortical structures and the hippocampus, and suggests the participation of the hippocampus in some absence seizures.

CONCLUSIONS

This study demonstrates the involvement of the hippocampus during PDs induced by GBL in rats. Hippocampal EEG became increasingly synchronous and coherent with EEG in the thalamus and neocortex, and hippocampal CA1 neurons fired consistently 40–80 ms prior to the peak of thalamic PDs. The results suggest that the hippocampus was entrained by thalamocortical PDs and may contribute to the cognitive deficits in patients with absence seizures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by Canadian Institutes of Health operating grant MOP 15685 to LSL, Ontario Brain Institute EpLink grant to LSL and SMM, and internal grants of Lawson Research Institute to SMM.

References

- Agathonikou A, Panayiotopoulos CP, Giannakodimos S, Koutroumanidis M. Typical Absence Status in Adults: Diagnostic and Syndromic Considerations. *Epilepsia*. 1998; 39:1265–1276. [PubMed: 9860061]
- Avoli M, Gloor P, Kostopoulos G, Gotman J. An analysis of penicillin-induced generalized spike and wave discharges using simultaneous recordings of cortical and thalamic single neurons. *J Neurophysiol*. 1983; 50:819–837. [PubMed: 6631465]
- Bai X, Vestal M, Berman R, Negishi M, Spann M, Vega C, Desalvo M, Novotny EJ, Constable RT, Blumenfeld H. Dynamic time course of typical childhood absence seizures: EEG, behavior, and functional magnetic resonance imaging. *J Neurosci*. 2010; 30:5884–5893. [PubMed: 20427649]
- Banerjee PK, Snead OC III. Thalamic mediodorsal and intralaminar nuclear lesions disrupt the generation of experimentally induced generalized absence-like seizures in rats. *Epilepsy Res*. 1994; 17:193–205. [PubMed: 8013443]
- Banerjee PK, Hirsch E, Snead OC. Gamma-Hydroxybutyric acid induced spike and wave discharges in rats: relation to high-affinity [3H]gamma-hydroxybutyric acid binding sites in the thalamus and cortex. *Neuroscience*. 1993; 56:11–21. [PubMed: 8232911]
- Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Mosche GW, Nordli D, Plouin P, Scheffer IE. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia*. 2010; 51:676–685. [PubMed: 20196795]
- Bertram EH, Zhang DX. Thalamic excitation of hippocampal CA1 neurons: a comparison with the effects of CA3 stimulation. *Neuroscience*. 1999; 92:15–26. [PubMed: 10392827]
- Buzsáki G. The thalamic clock: emergent network properties. *Neuroscience*. 1991; 41:351–364. [PubMed: 1870695]

- Buzsáki G, Leung LWS, Vanderwolf CH. Cellular bases of hippocampal EEG in the behaving rat. *Brain Res Rev.* 1983; 6:139–171.
- Caplan R, Siddarth P, Stahl L, Lanphier E, Vona P, Gurbani S, Koh S, Sankar R, Shields WD. Childhood absence epilepsy: behavioral, cognitive, and linguistic comorbidities. *Epilepsia.* 2008; 49:1838–1846. [PubMed: 18557780]
- Carr DB, Sesack SR. Hippocampal afferents to the rat prefrontal cortex: synaptic targets and relation to dopamine terminals. *J Comp Neurol.* 1996; 369:1–15. [PubMed: 8723699]
- Coenen AML, Drinkenburg WHIM, Inoue M, van Luijckelaar ELJM. Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. *Epilepsy Res.* 1992; 12:75–86. [PubMed: 1396543]
- Crunelli V, Leresche N. Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci.* 2002; 3:371–382. [PubMed: 11988776]
- Dolleman-Van der Weel MJ, Lopes da Silva FH, Witter MP. Nucleus reuniens thalami modulates activity in hippocampal field CA1 through excitatory and inhibitory mechanisms. *J Neurosci.* 1997; 17:5640–5650. [PubMed: 9204945]
- Gloor P. Generalized cortico-reticular epilepsies: some considerations on the pathophysiology of generalized bilaterally synchronous spike and wave discharge. *Epilepsia.* 1968; 9:249–263. [PubMed: 4975028]
- Guidotti A, Ballotti PL. Relationship between pharmacological effects and blood and brain levels of gamma-butyrolactone and gamma-hydroxybutyrate. *Biochem Pharmacol.* 1970; 19:883–894. [PubMed: 5507693]
- Inoue M, Duysens J, Vossen JM, Coenen AM. Thalamic multiple-unit activity underlying spike-wave discharges in anesthetized rats. *Brain Res.* 1993; 612:35–40. [PubMed: 8330210]
- International League Against Epilepsy (ILAE). Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia.* 1989; 30:389–399. [PubMed: 2502382]
- Jackson DC, Dabbs K, Walker NM, Jones JE, Hsu DA, Stafstrom CE, Seidenberg M, Hermann BP. The neuropsychological and academic substrate of new/recent-onset epilepsies. *J Pediatr.* 2013; 162:1047–1053. [PubMed: 23219245]
- Kandel A, Buzsáki G. Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. *J Neurosci.* 1997; 17:6783–6797. [PubMed: 9254689]
- Kandel A, Bragin A, Carpi D, Buzsáki G. Lack of hippocampal involvement in a rat model of petit mal epilepsy. *Epilepsy Res.* 1996; 23:123–127. [PubMed: 8964273]
- Leung LS. Orthodromic activation of the hippocampal CA1 region in the rat. *Brain Res.* 1979; 176:49–63. [PubMed: 487183]
- Leung, LS. Field potential generation and current source density analysis. In: Vertes, RP., Stackman, RW., editors. *Electrophysiological Recording Techniques*. Humana Press Clifton; New Jersey: 2010. p. 1-26.
- Leung LS, Yu H. Theta-frequency resonance in hippocampal CA1 neurons in vitro demonstrated by sinusoidal current injection. *J Neurophysiol.* 1998; 79:1592–1596. [PubMed: 9497437]
- Leung LS, Luo T, Ma J, Herrick I. Brain areas that influence general anesthesia. *Prog Neurobiol.* 2014; 122:24–44. [PubMed: 25172271]
- Leung LW, Lopes da Silva FH, Wadman WJ. Spectral characteristics of the hippocampal EEG in the freely moving rat. *Electroencephal Clin Neurophysiol.* 1982; 54:203–219.
- Leung LWS, Buzsáki G. Spectral analysis of hippocampal unit train in relation to hippocampal EEG. *Electroencephal Clin Neurophysiol.* 1983; 56:668–671.
- Liu Z, Vergnes M, Depaulis A, Marescaux C. Involvement of intrathalamic GABAB neurotransmission in the control of absence seizures in the rat. *Neuroscience.* 1992; 48:87–93. [PubMed: 1316571]
- Ma J, Shen B, Stewart LS, Herrick IA, Leung LS. The septohippocampal system participates in general anesthesia. *J Neurosci.* 2002; 22:RC200, 1–6. [PubMed: 11784812]
- Markand ON. Lennox-Gastaut syndrome (childhood epileptic encephalopathy). *J Clin Neurophysiol.* 2003; 20:426–41. [PubMed: 14734932]

- Martin JH. Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. *Neurosci Lett*. 1991; 127:160–164. [PubMed: 1881625]
- Meeren HK, Pijn JP, van Luijtelaar EL, Lopes da Silva FH. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci*. 2002; 22:1480–1495. [PubMed: 11850474]
- Meeren H, van Luijtelaar G, Lopes da Silva F, Coenen A. Evolving concepts on the pathophysiology of absence seizures: the cortical focus theory. *Arch Neurol*. 2005; 62:371–376. [PubMed: 15767501]
- Nehlig A, Vergnes M, Boyet S, Marescaux C. Local cerebral glucose utilization in adult and immature GAERS. *Epilepsy Res*. 1998; 32:206–212. [PubMed: 9761321]
- Onat FY, van Luijtelaar G, Nehlig A, Snead OC. The involvement of limbic structures in typical and atypical absence epilepsy. *Epilepsy Res*. 2013; 103:111–123. [PubMed: 22989853]
- Paxinos, G., Watson, C. The rat brain in stereotaxic coordinates. 6. Academic Press; New York: 2009. Compact
- Perez Velazquez JL, Huo JZ, Dominguez LG, Leshchenko Y, Snead OC. Typical versus atypical absence seizures: network mechanisms of the spread of paroxysms. *Epilepsia*. 2007; 48:1585–1593. [PubMed: 17484751]
- Polack PO, Guillemet I, Hi E, Deransart C, Depaulis A, Charpier S. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci*. 2007; 27:6590–6599. [PubMed: 17567820]
- Polack PO, Mahon S, Chavez M, Charpier S. Inactivation of the somatosensory cortex prevents paroxysmal oscillations in cortical and related thalamic neurons in a genetic model of absence epilepsy. *Cereb Cortex*. 2009; 19:2078–2091. [PubMed: 19276326]
- Semba K, Szechtman H, Komisaruk BR. Synchrony among rhythmical facial tremor, neocortical ‘alpha’ waves, and thalamic non-sensory neuronal bursts in intact awake rats. *Brain Res*. 1980; 195:281–298. [PubMed: 7397502]
- Shaw FZ. Is spontaneous high-voltage rhythmic spike discharge in Long Evans rats an absence-like seizure activity? *J Neurophysiol*. 2004; 91:63–77. [PubMed: 12826656]
- Shaw FZ. 7–12 Hz high-voltage rhythmic spike discharges in rats evaluated by antiepileptic drugs and flicker stimulation. *J Neurophysiol*. 2007; 97:238–247. [PubMed: 17035363]
- Snead OC. γ -Hydroxybutyrate model of generalized absence seizures: further characterization and comparison, with other absence models. *Epilepsia*. 1988; 29:361–368. [PubMed: 3391142]
- Snead OC, Depaulis A, Vergnes M, Marescaux C. Absence epilepsy: advances in experimental animal models. *Adv Neurol*. 1999; 79:253–278. [PubMed: 10514819]
- Steriade M, Contreras D, Amzica F. Synchronized sleep oscillations and their paroxysmal developments. *Trends Neurosci*. 1994; 17:201–208.
- Tenney JR, Duong TQ, King JA, Ludwig R, Ferris CF. Corticothalamic Modulation during Absence Seizures in Rats: A Functional MRI Assessment. *Epilepsia*. 2003; 44:1133–1140. [PubMed: 12919383]
- Tolmacheva EA, van Luijtelaar G. Absence seizures are reduced by the enhancement of GABA-ergic inhibition in the hippocampus in WAG/Rij rats. *Neurosci Lett*. 2007; 416:17–21. [PubMed: 17280780]
- Tolmacheva EA, van Luijtelaar G, Chepurinov SA, Kaminski Y, Mares O. Cortical and limbic excitability in rats with absence epilepsy. *Epilepsy Res*. 2004; 62:189–198. [PubMed: 15579307]
- Vanderwolf CH. Neocortical and hippocampal activation in relation to behavior: effects of atropine, eserine, phenothiazines, and amphetamine. *J Comp Physiol Psych*. 1975; 88:300–323.
- Venzi M, Di Giovanni G, Crunelli V. A critical evaluation of the gamma-hydroxybutyrate (GHB) model of absence seizures. *CNS Neurosci Ther*. 2015; 21:123–140. [PubMed: 25403866]
- Vergnes M, Marescaux C, Depaulis A. Mapping of spontaneous spike and wave discharges in Wistar rats with genetic generalized non-convulsive epilepsy. *Brain Res*. 1990; 523:87–91. [PubMed: 2207693]
- Vergnes M, Marescaux C. Cortical and thalamic lesions in rats with genetic absence epilepsy. *J Neural Transm Suppl*. 1992; 35:71–83. [PubMed: 1512595]

- Vertes RP, Hoover WB, Szigeti-Buck K, Leranath C. Nucleus reuniens of the midline thalamus: link between the medial prefrontal cortex and the hippocampus. *Brain Res Bull.* 2007; 71:601–609. [PubMed: 17292803]
- Westmijse I, Ossenblok P, Gunning B, van Luijtelaar G. Onset and propagation of spike and slow wave discharges in human absence epilepsy: aMEG study. *Epilepsia.* 2009; 50:2538–2548. [PubMed: 19519798]

Figure 1.

Time-frequency plots of local field potentials (LFPs) during bursting and continuous paroxysmal discharges (PDs) induced by 200 mg/kg i.p. gamma-butyrolactone (GBL) in a representative rat. **A.** Time-frequency spectral plot (spectrogram) of the LFP at the ventrolateral (VL) thalamus, shown underneath spectrogram, during baseline awake-immobility, bursting PDs and continuous PDs. The spectrogram shows the distribution of power across frequency for each second of the 32-s segment of LFP; logarithmic power is coded in color, as shown by the calibration bar (right). During baseline, an episode of high voltage spindles (HVS) occurred with main frequency (dark red) at ~8 Hz. Bursts of high-amplitude PDs of 4–6 Hz occurred during the bursting PD stage, and relative constant amplitude of LFPs of 3–5 Hz occurred during the continuous PD stage. **B.** LFPs traces expanded to show baseline traces at different electrodes (cortex, CTX), with same episode of HVS and PD (red underline) labelled as in A. **C.** Time-expanded trace of the baseline HVS and bursting stage PD showing sharp (spiky) LFPs at the frontal cortex and VL thalamus, particularly at initiation of HVS and PD.

Figure 2.

Changes in power and coherence (group average) between local field potentials following GBL injection. **A.** Group averaged logarithmic power spectra of local field potentials from three selected brain areas, frontal cortex, ventrolateral (VL) thalamus (thal), and hippocampus (HPC); each spectrum was an average of 8 rats. Power shown with downward standard error of the mean; baseline awake-immobility periods excluded visually detectable high voltage spindles. Bursting stage of paroxysmal discharge (PD), at ~4 min after injection of 200 mg/kg i.p. GBL, was associated with increased peak power at ~5 Hz as compared to baseline immobility. Statistically significant difference between bursting and baseline power at a digital frequency (see Methods) is indicated by a circle (P). **B.** Power spectra during the continuous PD stage (~9 min after injection), compared to those during the bursting stage, with statistically significant difference between bursting and continuous stage power indicated by a circle (P). Power peak during the continuous PD stage shifted to ~3 Hz, with increased 1-Hz slow activity, as compared to the bursting PD stage. **C.** Three group-average coherence spectra, from left to right – frontal cortex and thalamus (FR-VL), thalamus and hippocampus (VL-HPC), and hippocampus-frontal (HPC-FR). For each panel, bursting PD stage were associated with a ~5-Hz coherence peak that decreased to a ~3 Hz peak during the continuous stage. Statistical difference from baseline coherence indicated by a red circle for the bursting PD stage, and a smaller blue circle for the continuous PD stage.

Figure 3.

Hippocampal neuronal firing in relation to GBL-induced thalamic paroxysmal discharges (PDs). **A.** Top, hippocampal multiple unit activity (ua) was recorded together with hippocampal (hpc) and thalamic (thal) local field potentials in a representative rat. A threshold (dashed horizontal line) was set to detect the highest negative peak of the thalamic PDs (bottom trace). **B.** At 5–8 min after GBL injection in a representative rat, amplitude-discriminated hippocampal unit firing in relation to triggered thalamic PD negative peak (37 sweeps) is shown as a peri-event time histogram (PETH), showing probability of firing per bin of 10 ms duration. Units fired significantly more than chance ($*P < 0.001$) at 65 ms before the thalamic PD negative peak. **C.** Same rat during baseline, hippocampal unit PETH triggered by the high-voltage spindle activity (of ~ 9 Hz) did not show significant firing in relation to the thalamic spindle peaks (18 sweeps). **D.** Group average PETH from 6 different hippocampal electrodes in 5 rats, at 4–9 min after GBL injection, plotted with mean plus standard error, showing significant higher unit activity firing at -25 to -95 ms before the negative peak of the thalamic PD (time zero).

Figure 4.

Spectral analysis of hippocampal unit activity in relation to thalamic local field potentials before and after GBL, selected for 3–9 Hz peak frequency. **A.** Hippocampal multiple unit activity (UA) and thalamic local field potentials (Thal LFP) during baseline (selected for HVS activity), and 9 and 13 min after GBL 200 mg/kg i.p. tUA = unit activity above threshold (horizontal line); fUA = filtered unit activity after anti-aliasing filter. **B.** Auto power spectra of the thalamic LFP (B1) and filtered “unit” activity (B2), and the coherence spectrum between LFP and unit activity (B3). Spectra for baseline and 9 min post-GBL are overlaid. A power peak (arrow) was found at 7.4 Hz during baseline, and at 4.9 Hz at 9 min after GBL. LFP-unit coherence peaks was found at ~4.7 Hz for both baseline (*, $P < 0.005$) and 9 min after GBL (**, $P < 0.001$). **C.** Same as B except spectra from 9 min and 13 min after GBL injection are overlaid. A decrease in peak power and coherence values occurred between 9 min and 13 min after GBL, but LFP-unit coherence remained significant (*, $P < 0.005$). Segments (selected for the dominant frequency) contributing to average spectra: baseline, 59; 9 min post-GBL, 57; 13 min post-GBL, 69.

Figure 5.

Duration of GBL-induced 2.5–6 Hz paroxysmal discharges (PDs) was shortened by hippocampal infusion of muscimol as compared to saline. A. Hippocampal (LH) and thalamic (LT) field potentials of a representative rat were recorded following (1) saline or (2) muscimol infusion into CA1 of the mid-hippocampus. Logarithmic power spectra of LH and LT field potentials, illustrated by traces on top, during four time periods from left to right: immobility baseline (Imm base), maximal PD power, last minute of PDs, and first minute with peak frequency of <2.5 Hz (evaluated by power spectrum at LH). Following saline infusion into the hippocampus, 2.5 – 6 Hz PDs were observed until 13 min after GBL

injection, while in the same rat following muscimol infusion (performed a week later), PDs were observed only until 5 min following GBL injection. B. Group data of average hippocampal peak frequency (error bars are one standard error of the mean) for 6 rats given both saline and muscimol infusion. During baseline (time <0), average peak frequencies of 8-Hz (during walking) and 4-Hz (awake immobility) were found. After GBL injection (time 0), walking theta was observed at 2 min, after which 2.5–6 Hz PDs dominated. The peak frequency after GBL injection was significantly different between pre-infusion with muscimol as compared to saline ($P<0.05$, 2-way ANOVA).

Table 1

Average peak coherence between local field potentials at two electrodes, in the 2.5 – 9 Hz range (n= 8 rats) during baseline, bursting and continuous paroxysmal discharges (PDs) induced by 200 mg/kg i.p. GBL injection. Bursting PDs occurred 4–9 min after, and continuous PDs 9–13 min after GBL injection.

Electrode Pairs	Baseline	Bursting PDs	Continuous PDs
Hippocampus-thalamus	0.49	0.78 [*]	0.71
Hippocampus-frontal cortex	0.28	0.55 [*]	0.51
Hippocampus-parietal cortex	0.39	0.72 [*]	0.71
Hippocampus-visual cortex	0.32	0.61 [*]	0.55
Thalamus-parietal cortex	0.39	0.69 [*]	0.56
Thalamus-frontal cortex	0.58	0.64	0.61
Thalamus-visual cortex	0.46	0.61	0.63
Frontal cortex-parietal cortex	0.41	0.52	0.45
Frontal cortex-visual cortex	0.52	0.53	0.50
Visual cortex-parietal cortex	0.47	0.51	0.46

Thalamus: ventrolateral thalamus. Hippocampus: dorsal CA1 stratum radiatum.

^{*} Significant increase in coherence relative to baseline ($P < 0.05$).