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The hippocampus participates in a pharmacological rat model of absence seizures

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ABSTRACT

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.

1. 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

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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 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.

2. Methods

2.1. Animals

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

2.2. Surgery and 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 μm 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 μm, was chronically implanted into the dorsal hippocampus (P 4.0, L 2.7). 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 P 4.6, L 2.5, V 3.0 (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.

2.3. 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 and 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 necortical and hippocampal

[1] The 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).