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Mechanism of highly synchronized bilateral hippocampal activity

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ABSTRACT

In vivo studies of epileptiform discharges in the hippocampi of rodents have shown that bilateral seizure activity can sometimes be synchronized with very small delays (<2 ms). This observed small time delay of epileptiform activity between the left and right CA3 regions is unexpected given the physiological propagation time across the hemispheres (>6 ms). The goal of this study is to determine the mechanisms of this tight synchronization with in-vitro electrophysiology techniques and computer simulations. The hypothesis of a common source was first eliminated by using an in-vitro preparation containing both hippocampi with a functional ventral hippocampal commissure (VHC) and no other tissue. Next, the hypothesis that a noisy baseline could mask the underlying synchronous activity between the two hemispheres was ruled out by low noise in-vivo recordings and computer simulation of the noisy environment. Then we built a novel bilateral CA3 model to test the hypothesis that the phenomenon of very small left-to-right propagation delay of seizure activity is a product of epileptic cell network dynamics. We found that the commissural tract connectivity could decrease the delay between seizure events recorded from two sides while the activity propagated longitudinally along the CA3 layer thereby yielding delays much smaller than the propagation time between the two sides. The modeling results indicate that both recurrent and feedforward inhibition were required for shortening the bilateral propagation delay and depended critically on the length of the commissural fiber tract as well as the number of cells involved in seizure generation. These combined modeling/experimental studies indicate that it is possible to explain near perfect synchronization between the two hemispheres by taking into account the structure of the hippocampal network.

Introduction

Epilepsy is the most common chronic neurological disease, affecting more than 50 million people worldwide (Duncan et al., 2006). It is characterized by intermittent bursts of aberrant electrical activity in the brain, resulting in seizure symptoms. Mesial temporal lobe epilepsy (MTLE), often accompanied by hippocampal sclerosis, is the most common and most medically refractory form of epilepsy (Avoli et al., 2002; King et al., 1995; Spencer, 2002). One quarter to one third of people with unilateral lesions and MTLE appear to have bilateral interictal spike foci (Gilmore et al., 1994; Gupta et al., 1973; Hughes, 1985; Richard et al., 1997). One of the tracts which could be responsible for transmitting seizure activity bilaterally to the contralateral hippocampus is the hippocampal commissure (Khalilov et al., 2003; Rosenzweig et al., 2011).

The human dorsal hippocampal commissure (DHC) is a sizable tract which travels between bilateral hippocampal formations (Colnat-Coulbois et al., 2010; Gloor et al., 1993; Rosenzweig et al., 2011). Depth electrode EEG recordings show that secondarily generalized MTLE occurs in the bilateral hippocampus before surrounding structures, suggesting the DHC is responsible for contralateral spread of seizures (Finnerty et al., 1993; Gloor et al., 1993; Rosenzweig et al., 2011). While the DHC is clearly present in humans (Colnat-Coulbois et al. 2010), it is somewhat small in rodents. The ventral hippocampal commissure (VHC) is the dominant hippocampal commissural tract in rodents (Bliss et al., 1983; Swanson et al., 1978; Witter and Amaral, 2004). This tract primarily interconnects CA3 cells to CA1 cells of the contralateral hippocampus (Bliss et al., 1983; Queiroz and Mello, 2007), which has been demonstrated in an after discharge model of epilepsy in rats showing activity crossing between two hippocampi through the VHC (Fernandes de Lima et al., 1990). Several researchers have reported that the activity in the two hemispheres can be highly synchronized (Allen et al., 1992; Kobayashi et al., 1994). In some cases, the delay between bilateral interictal or ictal events can be very small (<2 ms) (Fernandes de Lima et al., 1990). This result is

Abbreviations: HCP, homotopic cell pairs, cells labeled with same number on both sides of the model.

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unexpected given the ~10 mm length of the VHC axon tract (George and Charles, 2007) and ~6 ms contralateral propagation delay of evoked field responses between the two hippocampi (Feng and Durand, 2005).

The observation of a <2 ms delay between left and right hippocampal activity is puzzling in that the timing does not conform to the model of seizure activity beginning in one hippocampus and traveling across the VHC to the contralateral hippocampus since a minimum delay of 6 ms would be expected. There are several hypotheses to explain this phenomenon: 1) a common third source that projects to both hippocampi and is equidistant from each; 2) noise present on both sides that could induce and/or mask synchronization; and 3) a new mechanism whereby the delay between the two sides is decreasing incrementally as the activity propagates along the length of the bilaterally connected CA3 layers. To test these hypotheses we used electrophysiological and computational modeling techniques. In particular, we have developed a slice preparation and a computer model that reproduces the high degree of synchrony observed in vivo. Preliminary results have appeared in abstract form (Wang et al., 2011).

Methods

In vivo surgical procedures

All procedures used in this study were approved by the Institutional Animal Care and Use Committee, Case Western Reserve University, Cleveland. Adult Sprague Dawley rats (300–350 g) were anesthetized with urethane (1.5 g/kg i.p.) and placed in a stereotaxic apparatus. Body temperature was maintained at 37 °C with a heating pad. As shown in Fig. 1a, several burr holes were drilled through the skull for placement of stimulation electrode (AP −1.0 mm, ML −0.5 mm), bilateral CA3 recording electrodes (AP −3.0 mm, ML ±3.0 mm), ground screw (AP −1.0 mm, ML 1.0 mm), reference screw (AP 3.0 mm, ML 2.0 mm) and micro-syringes/CA1 recording electrodes (AP −5.0 mm, ML ±4.0 mm). All locations were relative to bregma. Accurate placement could be confirmed by recording antidromic evoked potentials in CA3 from VHC stimulation (Tang and Durand, 2012). Artificial cerebrospinal fluid (ACSF) was warmed to 37 °C and applied to the exposed skull. Normal ACSF consisted of the following (in mM): 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1.5 $MgSO_4$, 26 $NaHCO_2$ and 2 g/L D-glucose.

To generate unilateral seizure activity, ACSF containing 25 mM 4-aminopyridine (4-AP) was injected into the CA1 region at a rate of 0.1 μl/min (Bahar et al., 2006; Tang and Durand, 2012). The injection was 1.0 μl at the start of the first hour and 0.5 μl during subsequent hours for a total of 7 h. Resultant epileptiform activity in CA3 was verified by hyperactive evoked potentials in this region as mentioned in the previous procedure.

For VHC stimulation, polyimide insulated tungsten electrodes (254 μm diameter, 1 mm tip exposure, A-M Systems, Carlsborg, WA, USA) were used. To record epileptic activity, recording electrodes (127 μm diameter, Parylene-C, A-M Systems) were positioned in both left and right hippocampal CA3 regions. Recorded signals were amplified 100 times by Model 1700 4-channel amplifiers (A-M Systems) with filter frequency ranging from 1 Hz to 5 kHz. Signals were then sampled at a rate of 20 kHz with an ML795 PowerLab/16SP data acquisition system (AD Instruments, Colorado Springs, CO, USA) and stored into a computer for off-line analysis.

In vitro experimental procedures

Adult Sprague Dawley (SD) rats (12–21 days) were anesthetized using ethyl ether or isofluorane and decapitated. The brain was removed and placed in cold (3–4 °C) oxygenated (O_2 95%, CO_2 5%) sucrose-rich ACSF. The cerebellum was detached and the ventral surface of the brain was secured in a vibrating-blade microtome (VT1000S, Leica, Buffalo Grove, IL, USA) containing sucrose-based cold, oxygenated ACSF. A novel bilateral hippocampal slice preparation was utilized. In this preparation, the bilateral hippocampi (including entorhinal cortices) and connecting VHC are preserved after other tissues have been carefully dissected away (Toprani and Durand, 2013). 750 μm axial slices were cut and immediately preserved in oxygenated ACSF for at least 60 min before being transferred to an interface-recording chamber (Harvard Apparatus, Holliston, MA, USA). Slice viability was confirmed by the presence of distinct, healthy cell layers marked by cresyl violet (CV) staining in select slices and by extracellular field recordings of evoked potentials from CA3 and CA1 larger than 1 mV for all preparations in ACSF that do not diminish over the course of the experiment. The axonal anatomy of the VHC was examined histologically for select slices using luxol fast blue (LFB), while the functional connection was established in all instances by bilateral extracellular evoked potentials that could be elicited by a single stimulus in the VHC tract (Fig. 1b). Evoked responses in ACSF averaged 3 mV ± 1.7 mV with a single vertex.

Epileptic activity was generated by injecting 4-AP in ACSF (100 μM) into the CA3 region with a micro-syringe. Effective seizure-like activity generation included regular interictal-like and ictal-like waveforms. Given the duration of interictal events recorded was 0.79 ± 0.22 s (visually identified, n = 100, from 4 rats), seizures were defined as high-frequency activity (>10 Hz) lasting at least 2 s with variable amplitude as for the in-vivo model.

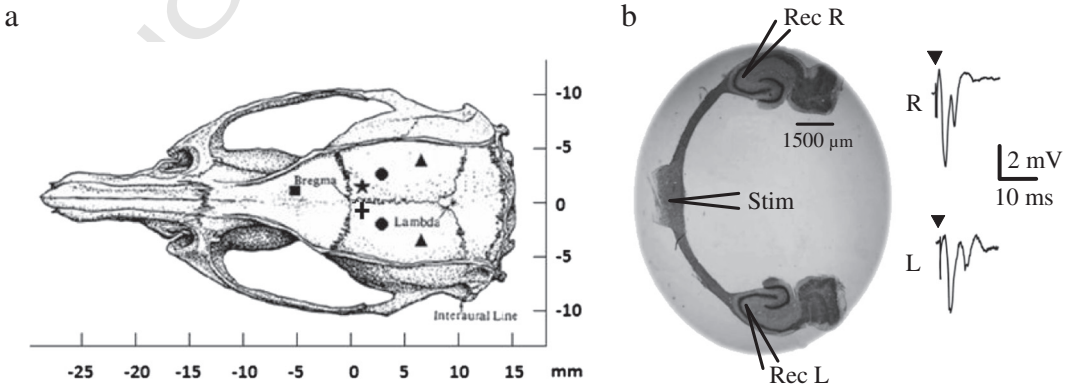

Fig. 1. In-vivo and in-vitro preparations a. Burr holes for in vivo placement of stimulation electrode (cross), CA3 recording electrodes (two dots), ground screw (pentagram), reference screw (square) and micro-syringe/CA1 recording electrodes (two triangles) (image modified from Tang and Durand, 2012). b. Bilateral hippocampal slice preparation developed with both hippocampi and connecting VHC still preserved after other tissues were carefully dissected away. To test the functional connection and measure the propagation time between left and right hippocampi, stimulation was applied in the middle of VHC axon tract with simultaneous left and right CA3 recordings (Rec L, Rec R) in ACSF solution (image modified from Toprani and Durand, 2013).