Published in final edited form as:

Exp Neurol. 2014 January ; 251: 101–111. doi:10.1016/j.expneurol.2013.11.014.

Mechanism of Highly Synchronized Bilateral Hippocampal Activity

Y. Wang^a, S. Toprani^b, Y. Tang^b, T. Vrabec^c, and D.M. Durand^{b,*}

^aDepartment of Biomedical Engineering, Zhejiang University, Hangzhou, Zhejiang, China

^bNeural Engineering Center, Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio, USA

^cDepartment of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio, USA

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

© 2013 Elsevier Inc. All rights reserved.

Address Correspondence to: Dr. Dominique M. Durand, Professor of Biomedical Engineering, Neuroscience, Physiology and Biophysics, Departments of Biomedical Engineering, Case Western Reserve University, Neural Engineering Center, Room 309 Wickenden Building 2071 Martin Luther King Jr. Drive Cleveland, OH 44106-7207, dxd6@case.edu, phone: 216 368 3974, fax: 216 368 4872, web: nec.cwru.edu.

Y. Wang is with Department of Biomedical Engineering, Zhejiang University, Room 217, Zhouyiqing Building, 38 Zheda Rd, Hangzhou, Zhejiang, 310027 China (kanewy@msn.com).

S. Toprani is with Neural Engineering Center, Department of Biomedical Engineering Case Western Reserve University, Cleveland OH 44106 USA (sct12@case.edu).

Y. Tang is with Neural Engineering Center, Department of Biomedical Engineering, Case Western Reserve University, Cleveland OH 44106 USA (dyt1@case.edu).

T. Vrabec is with Department of Biomedical Engineering, Case Western Reserve University, Cleveland OH 44106 USA (tina.vrabec@case.edu).

D.M. Durand is with Neural Engineering Center, Department of Biomedical Engineering, Case Western Reserve University, Cleveland OH 44106 USA (dxd6@case.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

possible to explain near perfect synchronization between the two hemispheres by taking into account the structure of the hippocampal network.

Keywords

Epilepsy; Synchronization; in vivo; in vitro; in silico

I. 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 et al., 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 et al., 2004). This tract primarily interconnects CA3 cells to CA1 cells of the contralateral hippocampus (Bliss et al., 1983; Queiroz et al., 2007), which has been demonstrated in an afterdischarge model of epilepsy in rats showing activity crossing between two hippocampi through the VHC (Fernandes 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 et al., 1990). This result is 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 travelling 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).

II. Methods

2.1. *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 $\pm$ 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 $\pm$ 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 NaH₂PO₄, 2 CaCl₂, 1.5 MgSO₄, 26 NaHCO₂ 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 μ l/min (Bahar et al., 2006; Tang and Durand, 2012). The injection was 1.0 μ l at the start of the first hour and 0.5 μ l during subsequent hours for a total of seven hours. 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 μ m diameter, 1 mm tip exposure, A-M Systems, Carlsborg, WA, USA) were used. To record epileptic activity, recording electrodes (127 μ m 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.

2.2. *In vitro* experimental procedures

Adult Sprague Dawley (SD) rats (12–21 days) were anesthetized using ethyl ether or isoflurane and decapitated. The brain was removed and placed in cold (3–4 °C) oxygenated (O₂ 95%, CO₂ 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 μ m axial slices were cut and immediately preserved in oxygenated ACSF for at least 60 minutes 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 $\pm$ 1.7 mV with a single vertex.

Epileptic activity was generated by injecting 4-AP in ACSF (100 μ M) 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.

Extracellular field potentials were recorded simultaneously from the CA3 stratum pyramidale of both hippocampi (Fig. 1 b) using glass microelectrodes (1–4 M Ω) filled with 150mM NaCl. All signals were amplified using an Axoclamp-2A microelectrode amplifier (Axon Instruments, Sunnyvale, CA, USA), low-pass filtered (5 kHz), and further amplified by a FLA-01 (Cygnus Technology, Delaware Water Gap, PA, USA), then stored on a DT-200 digital tape recorder (Microdata Instrument, S. Plainfield, NJ, USA) as well as on computer via an optical data acquisition program (44.1 kHz sampling rate, Audio Companion, Roni Music).

2.3. Modeling of bilateral CA3 network

The model was designed to simulate the interaction between two pools of CA3 neurons interconnected by the VHC (Fig. 2a) in the presence of an epileptogenic agent such as 4-AP. Fig. 2b shows the main diagram of the model containing 200 neurons on either side. Two columns of neurons in the middle (L1, L11...L191 and R1, R11...R191) are connected to both contralateral and ipsilateral neurons along the longitudinal direction, and the rest are connected only within the ipsilateral side. The distance between the two populations (length of the VHC) was 10 mm (George and Charles, 2007). While the contralateral connections were kept the same, four different types of ipsilateral networks were studied to determine the effect of the network configuration on the synchronization and the delay, which were: “Random” (Fig. 2c), “In-Outward” (Fig. 2d), “All-Outward” (Fig. 2e) and “All-Inward” (Fig. 2f) connection respectively. Except for the “Random” network, both transverse and longitudinal connections of the cell layers were implemented symmetrically in two sides. In the longitudinal direction, a single direction of propagation was implemented since activity was observed to propagate preferentially from septal lobe to temporal lobe (Kibler and Durand, 2011; Zhang et al., 2011). Different combinations of inward and outward connections in the transverse direction were implemented in the four ipsilateral networks, which were used to simulate the physiological recurrent and feedforward connections in the CA3 region respectively (Kali and Dayan, 2000; Laurberg, 1979; Traub and Wong, 1982).

Individual neurons were modeled by a soma and 2 dendrites (basal and apical) with one excitatory synaptic input on each and two axonal branches with excitatory synapses. These cells are known to have a single axon with early branching. This was modeled as 2 axons for simplicity (Tamamaki et al., 1987; Tamamaki and Nojyo, 1990). 4-AP is a potent potassium channel blocker which generates significant excitation (Pena and Tapia, 2000; Perreault and Avoli, 1991; Wu et al., 2009), the neurons in the network can be seen as numerous pyramidal-interneuron combinations, and their mutual effect (output) during seizure is still excitation. Therefore to simplify the problem, only excitatory neurons (pyramidal neuron) were included in this model. To initiate seizure activity, we applied stimulation unilaterally to the first cell that has both ipsilateral and contralateral connections but stimulation of other cells could also generate similar activity.

MATLAB (The MathWorks, Natick, MA, USA) was used to generate neuron locations on each side randomly but within a specified 2D layer which was 0.6 mm longitudinally by 0.3 mm transversely on each side (Anderson et al., 2009; George and Charles, 2007). Previous CA3 modeling studies used a variety of network sizes (N) from 100 neurons to 3000 neurons (Netoff et al., 2004; Traub and Wong, 1982). The number of neurons (N) was 200

for most simulations but other network sizes were also tested to test the robustness of the model.

Each neuron was modeled by using the software package NEURON (Carnevale and Hines, 2006; Hines and Carnevale, 2001) with the properties described and parameters shown in Table 1 (C_m is the membrane specific capacitance, R_i is the axoplasmic specific resistance and C_e is the extracellular specific conductivity. Length of the axon D_n is determined as the distance between two connecting neurons with equation:

$$D_n = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}, \quad [1]$$

where x and y are the neuron's coordinates and varied between 0 mm and 10 mm). The synaptic delay was set at 1 ms (Traub et al., 1991), and synaptic weight was randomly generated within 0.1 to 0.8. Only Hodgkin-Huxley type channels were included in this model. The time step of the simulation was 0.5 ms. The output from the NEURON simulation was imported into MATLAB to calculate the extracellular voltage that would be recorded by a recoding electrode in the center of each cell pool.

2.4. Calculation of extracellular field potentials

The extracellular activity was calculated by summing up the contribution from the compartmental activity of each neuron according to the distance between the neuron and recording site over time. Each compartment was considered to be a point source for extracellular recording. The recording electrodes were assumed to be located at the center of either the left or right populations (Fig. 2b). The extracellular voltages were calculated using following equation:

$$V_e = \sum_{i=1}^N \frac{i_{mi}}{4\pi\sigma_e r_i}, \quad [2]$$

where

V_e = extracellular voltage at the recording electrode

i_{mi} = the membrane current of each compartment

σ_e = the extracellular conductivity

r_i = the distance from the each compartment to the recording electrode

i = number of the compartments of all neurons

Equation [1] was used to calculate the contribution to the extracellular voltage from each compartment at the location of the recording electrode. Neurons within 50 μm to the recording site were excluded due to the recording limitation of the electrode in the *in vivo* environment (Buzsaki, 2004).

The bilateral delay was calculated by using cross-correlation, which was performed on the onset section of the ictal data segments (Tang and Durand, 2012). These segments were detected by visually analyzing the raw signal traces and spectrum (LabChart software, AD Instruments, Colorado Springs, CO, USA). Activities with frequency from 5 to 20 Hz and duration longer than 2 s were collected as ictal waveforms (Levesque et al., 2013). And the onset section was defined as the first large potential fluctuation of the ictal segment. For each onset section $x(n)$, $n = 1:N$, the time shifted correlation coefficients $CC(i)$, $i = 1:I$,

between x and the recording from the opposite side of the hippocampus $y(k)$, $k = 1:K$ (K is the number of data points from 100 s of recording) were computed as

$$CC(i) = \frac{\sum_{n=1}^N (x(n) - \bar{x})(y(i+n) - \bar{y})}{\sqrt{\sum_{n=1}^N (x(n) - \bar{x})^2} \sqrt{\sum_{n=1}^N (y(i+n) - \bar{y})^2}} \quad [3]$$

where $i=1:I$, $N=2000$, $K=2*10^6$, $I=K-N+1$,

$$\bar{y} = \frac{1}{2} \sum_{n=1}^N y(i+n) \quad [4]$$

In this way, the onset of each ictal segment was compared against every possible segment on the opposite side.

To test the hypothesis that the noise could mask synchronization, we implemented “Random” ipsilateral connection. Gaussian noise was directly added to the extracellular voltage signal to simulate a noisy environment. To simulate endogenous noise, Gaussian noise was added to cell body during the NEURON simulation.

III. Results

3.1. Bilateral synchronization during seizure activity *in vivo*

In order to determine the propagation time delay across the commissural fiber tract, single pulse stimulation was applied on the left or right CA3 and the resulting evoked potentials was recorded from the right or left CA3. The delay was measured to be 6.7 ± 1.9 ms ($n = 10$, Fig. 3a) and was similar to the *in vivo* values reported in the literature (Feng and Durand, 2005).

To measure the delay between epileptic events across the hemispheres, the potassium channel blocker 4-AP (25Mm) was applied unilaterally to induce epileptic activity. Seizures started on the injected side and appeared on the contralateral side within 10 min. Fig. 3b shows a 100 s example of bilateral epileptic signal recorded on the left (L) and right (R) sides. The ictal activity induced by 4-AP was continuous and robust, and it lasted throughout the length of the experiments. Amplitude of these activities varied from 2 mV to 10 mV. Two zoomed-in waveforms as well as a 50 Hz low-passed waveform (Fig. 3c and d) exhibit a high degree of synchronization in the time domain, mainly from the low frequency component. The onset section of each ictal event appeared as a large potential fluctuation with a steep ascending phase, and followed by a slowly descending phase (low frequency component) containing many high frequency spikes. However, the delay between the left onset and right onset was very low, it was difficult to discriminate the propagating direction of the seizure activity. Fig. 3e plots the maximum CC for each onset section and the absolute delay between the time of maximum correlation and the beginning of the section, and the mean value for 51 sections was found to be: 3.66 ± 2.2 ms. Fig. 3e shows a cumulative probability histogram of the bilateral delays (in absolute value). The results indicate that 92% of the delays are smaller than the time it takes to travel from one side to the other. One possible explanation for this discrepancy is a common source located at equal distance from both hippocampi that could be generating these synchronous events.

3.2. Highly synchronized activity is still observed in an *in vitro* preparation

To test the hypothesis that a third source could generate two highly synchronized signals with small delays in bilateral hippocampi, a novel preparation that preserved the two

hippocampi still connected by the VHC with all other tissue removed was developed (Toprani and Durand, 2013). To test the functional connection and measure the propagation time between left and right hippocampi, stimulation was applied in the middle of the VHC tract with simultaneous left and right CA3 recordings (Fig. 4a). In Fig. 4b, the left and right CA3 evoked potentials to VHC stimulation show that a complete interhemispheric propagation takes 10.0 ± 2.7 ms in this preparation ($n = 10$).

Epileptiform activity was then induced by adding $100 \mu\text{M}$ 4-AP into the ACSF. An example of 200 s recorded activity from the two interconnected hippocampi is shown in Fig. 4c. Epileptic activity were recorded 1 ~ 4 hours after bath application of 4-AP with variable field potential amplitudes from 5 to 10 mV. In general, the 4-AP induced epileptic activity was made of multiple ictal events, interrupted by periods of interictal event. Ictal event occurred every 1 ~ 3 min, while the interictal event occurred every 2 ~ 8 s. This activity appeared highly synchronized and resembled the bilateral activity recorded *in vivo*. The onset section also contained a large potential fluctuation with a steep ascending phase. The zoomed-in waveforms as well as the cross-correlation analysis (Fig. 4d, e and f) show the very low delay between the onset of left and right ictal events still exists in this preparation with potential third sources removed. Fig. 4f shows a cumulative probability histogram of the delay for 75 bilateral seizure events, indicating that 72% of the bilateral events have a delay smaller than the physiological propagation delay 10.0 ± 2.7 ms. Mean value of delays is 7.9 ± 5.3 ms.

Severing the VHC pathways destroyed the synchronization, indicating that the VHC was responsible for the synchronization (Toprani and Durand, 2013). In addition, recordings obtained from the VHC itself showed that these axons did not fire before the CA3 neurons, thereby eliminating the possibility that the source was within the VHC axons (data not shown).

Since the synchronized neural signals obtained in the bilateral preparations were solely from both hippocampi, the bilateral synchrony was intrinsic to this preparation. Therefore these results exclude the possibility that the synchrony was generated by a third common source.

3.3. Additive and endogenous noise do not explain the small delay

We then tested the hypothesis that the synchronization could take place between the two sides while the neural signal amplitudes were below the noise level and could not be observed until large signal amplitudes were reached. A computer model of the bilateral hippocampi connected by the VHC was implemented, using “Random” ipsilateral connections network shown in Fig. 2c. Virtual electrical stimulation applied to one cell on either side produced a bilateral seizure-like activity as shown in Fig. 5a, while stimulation of all cells on the left side and recording of evoked activity on the right side revealed a propagation time through the VHC that was 7 ms (Fig. 5b). In Fig. 5c, a significant delay between the two sides can be observed from the zoomed-in signals. The delay was also measured with cross-correlation and was found to be 7.0 ± 0.2 ms ($n = 5$). Different levels of noise were added to the extracellular voltage to simulate recording additive noise. SNR was determined by following equation:

$$SNR = \frac{P_{signal}}{P_{noise}} = \left(\frac{A_{signal}}{A_{noise}} \right)^2, \quad [5]$$

where A is the root mean square amplitude.

With additive noise ($SNR < 30$ dB, Fig. 5d), the bilateral delay appeared to be less than 2 ms, thereby giving the appearance that activity between the two sides was synchronized.

Fig. 5f shows a plot of the delay as a function of the SNR. At low noise level (SNR > 40 dB), the delay remained clearly detectable, from 6 to 8 ms. As the SNR decreased to less than 30 dB, the bilateral delays decreased significantly, suggesting that noise could mask the synchronization between two sides. We then measured the SNR of epileptiform activity obtained from *in vivo* experiments. Fig. 5e shows an example of such event with a high SNR. The mean SNR for 100 such events was measured to be 42 ± 5 dB. This SNR value was compared to the simulation result (arrow in Fig. 5f), indicating that the experimental noise level was insufficient to yield apparent small delays and therefore the noise hypothesis was abandoned.

3.4. Small time delays can be generated by longitudinal propagation along connected layers

We then investigated the effect of the network structure on the synchrony and the bilateral delay. In particular, we tested the hypothesis that the synchronization between the two connected sides could be enhanced as the activity propagated longitudinally along the CA3 layer, thereby generating small delays after a given time period. We first tested the model with “In-Outward” ipsilateral network (Fig. 2d) because the network included known features such as longitudinal propagation, transverse recurrent (inward) and feedforward (outward) connection.

Contralateral propagation time in this network was measured by stimulating all the cells on one side simultaneously and recording the evoked responses on the other side. A delay of 7 ms, similar to the *in vivo* experiments, was obtained (Fig. 6a). Epileptiform activity was simulated by stimulating the first cell on the left side (L1) (Fig. 2b). The model generated a 2s bilateral epileptic event with 25 bursts, each lasting about 50 ms (Fig. 6b). The bilateral delay at the onset of the epileptiform activity was calculated and was equal to the propagation delay (Fig. 6c left). However, as ongoing activity travelled back and forth and along the CA3 layers, the delay decreased from 7 ms to < 1.5 ms (Fig. 6c middle and right). The epileptiform signals recorded on both sides had similar shape, and their amplitudes did not change much before the seizure self-terminated. Depending on the size of the network, the entire epileptic events could last from 0.3 to 10 s, while the duration of each burst varied from 10 to 200 ms. Results from 50 simulations with randomly generated synaptic weights showed that the bilateral delay at the end of epileptic activity was 1.8 ± 0.6 ms, which demonstrated the model’s capability to generate the small delays observed experimentally.

We then analyzed the synchronization process by tracing unit activity of the homotopic cell pairs (HCP, cells labeled with same number on both sides in Fig. 2b). A histogram of delays between the two neurons of each HCP during different phases of epileptic activity is shown in Fig. 6d. The delays between HCPs decreased from 7 ms (mean value) at the onset of the activity to 1.5 ms at the end, showing all the cells on both sides were getting more synchronized over time. We also examined HCPs along the longitudinal direction. Fig. 6e shows the time of discharge of different HCPs throughout one burst. At the beginning, delay between L1 and R1 was equal to the physiological delay 7 ms. However, as the activity propagated longitudinally, the delay decreased gradually to as small as 0.5 ms (L61 and R61). This result indicates that the decrease in delay was correlated with the propagation in the longitudinal direction. The model’s prediction that it takes time for the interhemispheric delay to decrease is also confirmed by our experimental data (delay at the beginning was 53.5 ± 4.9 ms, $n = 25$) and the time required to reach tight synchronization was 28.1 ± 23.4 s ($n = 25$, data not shown). Similar results were obtained *in vitro* (delay at the beginning is 51.3 ± 2.7 ms, $n = 25$) (Khalilov’s et al, 2003).

To determine the relative role of ipsilateral recurrent and feedforward connections in generating the synchrony, we tested three other configurations: “Random” (Fig. 2c), “All-

Outward” (Fig. 2e) and “All-Inward” (Fig. 2f) respectively. We also studied the effect of these transverse connections quantitatively by changing the ratio of the inward and outward connections. This was done by adjusting the number of either inward or outward connection rows. For each ratio setting, 20 simulations were performed with randomly generated synaptic weights. In Fig. 7a, the “Random” network generated non-periodical high frequency activity with no synchronization between the two sides. The “All-Outward” structure exhibited periodical seizure-like activity with constant 8 ms delay (Fig. 7b). For the “All-Inward” structure, due to lack of “outward” pathway, the two columns of cells in the middle are unable to propagate their excitatory status to neighboring cells. This yielded small amplitude spike-like activity with a delay of 5 ± 1.2 ms (Fig. 7c). Therefore both inward and outward connections are required to generate the tight synchronization and small delays. By changing the ratio of inward to outward connection in the “In-outward” network, it was possible to determine the optimum structure for producing the very small delay. As shown in Fig. 7d, small delays (< 2 ms) can be achieved with a ratio from 1/4 to 2, with minimum delay appearing with ratio of 1. Therefore a similar amount of inward and outward connections is required to generate the smallest bilateral delay.

3.5. Robustness test of the model to produce small delay

We next tested the effect of two other parameters of the network: 1) length of the connection between the two hemispheres and 2) size of the network to determine the robustness of the tight bilateral synchronization achieved in the “In-Outward” network. The length of the VHC was varied from 5 mm to 15 mm (Fig. 8a). The simulations showed that the network was able to generate small delays (< 2 ms) only for interhemispheric distance values between 6 and 11 mm (Fig. 8b). These values are consistent with the anatomical range in rat brain sizes (George and Charles, 2007).

The size of the network was determined by the number of cells in both longitudinal direction (m) and transverse direction (n). A 3-Dimensional chart was constructed to show which combination of m and n could generate bilateral small delays. Based on 360 simulations (5 for each combination), two connected hippocampi could synchronize over a wide range of network sizes (height of bar < 2 ms, Fig. 8d). However a minimum of 6 layers and a maximum of 200 layers in the longitudinal direction were required to generate high synchronization between the two sides. For $m > 6$, increasing the size of the network decreased the delay, but too large networks ($m > 200$) prevented tight synchronization. Similarly, if the ipsilateral propagating pathway in transverse direction became too long ($n > 10$), the model could not yield small delays. Taken together, the results indicate that if either the distance between two hemispheres is too long or the bilateral network is too large (for the same bilateral tract), the two hemispheres cannot generate highly synchronized activity.

IV. DISCUSSION

The results of this study show that synchronization with very small delays across hemisphere (1) could be replicated in 4-AP induced epileptic rat model *in vivo*, *in vitro* and *in silico*; (2) that these surprisingly small delays cannot be explained by a third common source; (3) cannot be explained by the presence of noise; (4) can be explained by the presence of a longitudinal network; (5) depend critically on a mixture of recurrent and feedforward connections; and (6) is robust against the length of the commissural pathways and the size of the networks.

The small delay between two hippocampi was first observed in an anesthetized acute rat preparation by Fernandes (Fernandes et al., 1990), who showed repetitive very small time delay between afterdischarge events in left and right hippocampi in response to unilateral tetanus stimulation. Nearly all afterdischarge events had very small time delays and the

synchronization could be eliminated by cutting the commissural tract. Khalilov also found very small delay (< 2 ms) when two intact hippocampi still connected by the VHC were placed in three independent chambers and with one hippocampus kainite-treated for seizure generation (Khalilov et al., 2003). We have also observed this small delay *in vitro* with a bilateral brain slice preparation. The synchronization could also be abolished by severing the commissure pathway (Toprani and Durand, 2013). Very tight synchronization was also observed *in vivo* with 4-AP induced epileptiform activity (Tang and Durand, 2012). Contrary to afterdischarge activity, seizure events recorded bilaterally *in vivo* and *in vitro* did not all have a small delays but a significant portion of them did. Nevertheless, this very tight synchronization is a robust phenomenon that is observed in various animal preparations and different models of epilepsy. In all cases, bilateral delays smaller than the propagation delays were consistently observed.

To understand the mechanism of this small delay, a common source (such as a cortical structure) capable of synchronizing the two hippocampi is an obvious mechanism. To test this possibility, we used a newly developed bilateral hippocampal slice preparation (Toprani and Durand, 2013). The preparation preserves the two hippocampi and the VHC but excludes the possible interference from any other brain structures. Seizure activity induced by 4-AP in this preparation was synchronized bilaterally and only a subset of events had a very small delay (< 2 ms). Nevertheless these results indicate that bilateral tight synchronization is possible within a single slice of the brain. It is also possible that the axons connecting the two sides could generate seizure activity which would then appear simultaneously on both sides. However, axons are not normally associated with seizure foci and extracellular recordings obtained directly from the axons within the VHC did not reveal any detectable synchronized activity. Therefore the hypothesis of a common source can be safely abandoned.

Due to the limitations of current experimental technology, it is not possible to experimentally investigate the mechanism of the synchronization by recording from a large number of coupled cells simultaneously. Therefore, computer simulation methodology was used to study the mechanisms underlying this small delay between two coupled generators. The fact that coupled periodic oscillators can become synchronized is well known (Mirollo and Strogatz, 1990). Although epileptic activity in our model is not periodic, but rather stochastic, coupled chaotic neurons can also be synchronized (Shuai and Durand, 1999). The size and the number of neurons of the network were determined according to physiological measurement or estimation (Anderson et al., 2009). Seizure activity has been observed to spread bidirectionally, but preferentially from septal lobe to temporal lobe (Kibler and Durand, 2011). In this study, a single direction of propagation was implemented to simplify the model. The CA3 connectivity in the transverse direction is less clear and the neurons were arranged in four different network configurations: “Random”, “In-Outward”, “All-Outward” and “All-Inward” in order to test the role played by physiological recurrent and feedforward connections in the CA3 region (Kali and Dayan, 2000; Laurberg, 1979; Traub and Wong, 1982). Our results show only the “In-Outward” connection can yield stable small delay, demonstrating the importance of longitudinal propagation as well as the transverse recurrent and feedforward connection to explain this effect.

The simulation result for “In-Outward” connection predicts that the bilateral delay starts with the known propagation delays and slowly decreases to small values less than 2 ms. This prediction of the model is confirmed by our own experiments and also those carried out in the intact bilateral hippocampal preparation (Khalilov et al., 2003). This agreement between the model and the experiments suggests that the model captured an important feature of the mechanism of small bilateral delay, namely a progressive time dependent process that slowly increases the synchronization between the two hemispheres.

To ensure the robustness of the model, we tested various values of the length of the VHC and the size of the network. For the length of VHC, the small delay can be stably achieved within a range of values (6 to 11 mm), which is consistent with the anatomy of rats. This result may explain why a small delay between seizures is not often observed in human MTLE patients (Eross et al., 2009), because the connection between two temporal lobes in the human brain is the dorsal hippocampal commissure (DHC), and is clearly longer than the VHC in rodents (Colnat-Coulbois et al., 2010). Nevertheless, small interhemispheric delays have been found to exist in primary bilateral synchrony (PBS) of human epilepsy (Gotman, 1981; Kobayashi et al., 1992). These sources are bilaterally symmetrical, with subcortical origin thought to be projecting to multiple cortical areas (Chang et al., 2009; Tükel and Jasper, 1952). It is not clear which pathways are involved and whether these cases share the same mechanism described in above model.

The size of the network also clearly affected the ability of the two hippocampi to synchronize. Since tight synchronization is a time dependent process, with networks that were too small ($m < 6$, Fig. 8d), the seizure terminated before the two sides had sufficient time to interact and produce small delays. On the other hand, with networks that were too large ($m > 200$, Fig. 8d), excessive amounts of neurons discharged simultaneously during seizure generation resulting in seizure episodes overlapping with each other and the delay could not be observed or measured.

The relevance of the mechanism of synchronization is important to explain data obtained in both animal and clinical studies. Seizures in patients with mesial temporal lobe epilepsy has been shown to cross over into contralateral hemisphere, and are traced from the seizure focus to the contralateral hippocampus before spreading further (Gloor et al., 1993). The DHC has been suggested as a candidate to explain rapid transmission of epileptic events in humans (Rosenzweig et al., 2011). Moreover, this small delay mechanism described above could explain how smaller groups of hippocampal cells with activity not detectable by surface EEG could still be tightly synchronized leading the generation of secondary epileptogenic mirror foci (Khalilov et al., 2003).

Acknowledgments

This work is supported by a NIH grant 5R01NS060757-03 and a grant from the China Scholarship Council.

Abbreviations

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

References

1. Allen PJ, Smith SJ, et al. Measurement of interhemispheric time differences in generalised spike-and-wave. *Electroencephalogr Clin Neurophysiol.* 1992; 821:81–84. [PubMed: 1370147]
2. Anderson, P.; Morris, R., et al. *The Hippocampus Book*. Oxford University Press; USA: 2009.
3. Avoli M, D'Antuono M, et al. Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. *Prog Neurobiol.* 2002; 683:167–207. [PubMed: 12450487]
4. Bahar S, Suh M, et al. Intrinsic optical signal imaging of neocortical seizures: the 'epileptic dip'. *Neuroreport.* 2006; 17(5):499–503. [PubMed: 16543814]
5. Bliss TV, Lancaster B, et al. Long-term potentiation in commissural and Schaffer projections to hippocampal CA1 cells: an in vivo study in the rat. *J Physiol.* 1983; 341:617–626. [PubMed: 6620191]

6. Buzsaki G. Large-scale recording of neuronal ensembles. *Nat Neurosci.* 2004; 75:446–451. [PubMed: 15114356]
7. Carnevale, NT.; Hines, ML. *The NEURON Book*. Cambridge, UK: Cambridge University Press; 2006.
8. Chang EF, Nagarajan SS, et al. Magnetic source imaging for the surgical evaluation of electroencephalography-confirmed secondary bilateral synchrony in intractable epilepsy. *J Neurosurg.* 2009; 1116:1248–1256. [PubMed: 19575574]
9. Colnat-Coulbois S, Mok K, et al. Tractography of the amygdala and hippocampus: anatomical study and application to selective amygdalohippocampectomy. *J Neurosurg.* 2010; 1136:1135–1143. [PubMed: 20450277]
10. Duncan JS, Sander JW, et al. Adult epilepsy. *Lancet.* 2006; 3679516:1087–1100. [PubMed: 16581409]
11. Eross L, Entz L, et al. Interhemispheric propagation of seizures in mesial temporal lobe epilepsy. *Ideggyogy Sz.* 2009; 629–10:319–325.
12. Feng Z, Durand DM. Propagation of low calcium non-synaptic induced epileptiform activity to the contralateral hippocampus in vivo. *Brain Res.* 2005; 10551–2:25–35.
13. Fernandes de Lima VM, Pijn JP, et al. The role of hippocampal commissures in the interhemispheric transfer of epileptiform afterdischarges in the rat: a study using linear and non-linear regression analysis. *Electroencephalogr Clin Neurophysiol.* 1990; 766:520–539. [PubMed: 1701120]
14. Finnerty GT, Jefferys JG. Functional connectivity from CA3 to the ipsilateral and contralateral CA1 in the rat dorsal hippocampus. *Neuroscience.* 1993; 561:101–108. [PubMed: 8232909]
15. George, P.; Charles, W. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; UK: 2007.
16. Gilmore R, Morris H 3rd, et al. Mirror focus: function of seizure frequency and influence on outcome after surgery. *Epilepsia.* 1994; 352:258–263. [PubMed: 8156943]
17. Gloor P, Salanova V, et al. The human dorsal hippocampal commissure. An anatomically identifiable and functional pathway. *Brain.* 1993; 116(Pt 5):1249–73. [PubMed: 8221057]
18. Gotman J. Interhemispheric relations during bilateral spike-and-wave activity. *Epilepsia.* 1981; 224:453–466. [PubMed: 7262051]
19. Gupta P, Dharampaul C, et al. Secondary epileptogenic EEG focus in temporal lobe epilepsy. *Epilepsia.* 1973; 144:423–426. [PubMed: 4521099]
20. Hines ML, Carnevale NT. NEURON: a tool for neuroscientists. *Neuroscientist.* 2001; 72:123–135. [PubMed: 11496923]
21. Holsheimer J. Electrical conductivity of the hippocampal CA1 layers and application to current-source-density analysis. *Exp Brain Res.* 1987; 672:402–10. [PubMed: 3622698]
22. Hughes JR. Long-term clinical and EEG changes in patients with epilepsy. *Arch Neurol.* 1985; 423:213–23. [PubMed: 3977651]
23. Kali S, Dayan P. The involvement of recurrent connections in area CA3 in establishing the properties of place fields: a model. *J Neurosci.* 2000; 2019:7463–7477. [PubMed: 11007906]
24. Khalilov I, Holmes GL, et al. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. *Nat Neurosci.* 2003; 610:1079–1085. [PubMed: 14502289]
25. Kibler AB, Durand DM. Orthogonal wave propagation of epileptiform activity in the planar mouse hippocampus in vitro. *Epilepsia.* 2011; 529:1590–1600. [PubMed: 21668440]
26. King D, Spencer SS, et al. Bilateral hippocampal atrophy in medial temporal lobe epilepsy. *Epilepsia.* 1995; 369:905–910. [PubMed: 7649130]
27. Kobayashi K, Ohtsuka Y, et al. Primary and secondary bilateral synchrony in epilepsy: differentiation by estimation of interhemispheric small time differences during short spike-wave activity. *Electroencephalogr Clin Neurophysiol.* 1992; 832:93–103. [PubMed: 1378385]
28. Kobayashi K, Nishibayashi N, et al. Epilepsy with electrical status epilepticus during slow sleep and secondary bilateral synchrony. *Epilepsia.* 1994; 355:1097–1103. [PubMed: 7925158]
29. Laurberg S. Commissural and intrinsic connections of the rat hippocampus. *J Comp Neurol.* 1979; 1844:685–708. [PubMed: 422759]

30. Levesque M, Salami P, et al. Temporal lobe epileptiform activity following systemic administration of 4-aminopyridine in rats. *Epilepsia*. 2013; 54(4):596–604. [PubMed: 23521339]
31. Mainen ZF, Joerges J, et al. A model of spike initiation in neocortical pyramidal neurons. *Neuron*. 1995; 156:1427–39. [PubMed: 8845165]
32. Major G, Larkman AU, et al. Detailed passive cable models of whole-cell recorded CA3 pyramidal neurons in rat hippocampal slices. *J Neurosci*. 1994; 148:4613–38. [PubMed: 8046439]
33. Mirollo RE, Strogatz SH. Synchronization of pulse-coupled biological oscillators. *SIAM J Appl Math*. 1990; 50:1645–62.
34. Netoff TL, Clewley R, et al. Epilepsy in small-world networks. *J Neurosci*. 2004; 2437:8075–8083. [PubMed: 15371508]
35. Pena F, Tapia R. Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus in vivo: role of glutamate- and GABA-mediated neurotransmission and of ion channels. *Neuroscience*. 2000; 1013:547–561. [PubMed: 11113304]
36. Perreault P, Avoli M. Physiology and pharmacology of epileptiform activity induced by 4-aminopyridine in rat hippocampal slices. *J Neurophysiol*. 1991; 654:771–785. [PubMed: 1675671]
37. Queiroz CM, Mello LE. Synaptic plasticity of the CA3 commissural projection in epileptic rats: an in vivo electrophysiological study. *Eur J Neurosci*. 2007; 2510:3071–3079. [PubMed: 17561820]
38. Richard JM, Michael JO, et al. The mirror focus phenomenon and secondary epileptogenesis in human epilepsy. *J Epilepsy*. 1997; 10(2):78–85.
39. Rosenzweig, et al. The dorsal hippocampal commissure: when functionality matters. *J Neuropsychiatry Clin Neurosci* 2011. 2011; 233:E45–8.
40. Safronov BV, Wolff M, et al. Excitability of the soma in central nervous system neurons. *Biophys J*. 2000; 786:2998–3010. [PubMed: 10827978]
41. Shuai JW, Durand DM. Phase synchronization in two coupled chaotic neurons. *Phys Lett A*. 1999; 264:289–297.
42. Spencer SS. Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia*. 2002; 433:219–227. [PubMed: 11906505]
43. Swanson LW, Wyss JM, Cowan WM. An autoradiographic study of the organization of intrahippocampal association pathways in the rat. *J Comp Neurol*. 1978; 181:681–715. [PubMed: 690280]
44. Tamamaki N, Abe K, et al. Columnar organization in the subiculum formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus. *Brain Res*. 1987; 4121:156–160. [PubMed: 3607449]
45. Tamamaki N, Nojyo Y. Disposition of the slab-like modules formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus. *J Comp Neurol*. 1990; 2914:509–519. [PubMed: 2329188]
46. Tang Y, Durand DM. A novel electrical stimulation paradigm for the suppression of epileptiform activity in an in vivo model of mesial temporal lobe status epilepticus. *Int J Neural Syst*. 2012; 223:1250006. [PubMed: 23627622]
47. Toprani S, Durand DM. Fiber tract stimulation can reduce epileptiform activity in an in vitro bilateral hippocampal slice preparation. *Exp Neurol*. 2013; 240:28–43. [PubMed: 23123405]
48. Traub RD, Wong RK. Cellular mechanism of neuronal synchronization in epilepsy. *Science*. 1982; 2164547:745–747. [PubMed: 7079735]
49. Traub RD, Wong RK, et al. A model of a CA3 hippocampal pyramidal neuron incorporating voltage-clamp data on intrinsic conductances. *J Neurophysiol*. 1991; 662:635–650. [PubMed: 1663538]
50. Tukel K, Jasper H. The electroencephalogram in parasagittal lesions. *Electroencephalogr Clin Neurophysiol*. 1952; 44:481–494. [PubMed: 12998596]
51. Wang Y, Toprani S, et al. A mechanism to explain zero-delay bilateral seizure synchronization. *Conf Proc IEEE Eng Med Biol Soc*. 2011; 2011:7286–7289. [PubMed: 22256021]
52. Witter, MP.; Amaral, DG. Hippocampal formation. In: Paxinos, G., editor. *The Rat Nervous System*. 3. Elsevier; San Diego: 2004. p. 637-703.

53. Wu ZZ, Li DP, et al. Aminopyridines potentiate synaptic and neuromuscular transmission by targeting the voltage-activated calcium channel beta subunit. *J Biol Chem.* 2009; 28452:36453–36461. [PubMed: 19850918]
54. Zhang M, Kibler A, Durand DM. Mapping 4-AP induced epilepsy propagation with a micro-electrode array in intact hippocampus in vitro. *Conf IEEE Eng Med Biol Soc.* 2011; 2011:621–624.

Highlights

1. Bilateral seizure activity can be synchronized with very small delays (< 2 ms).
2. The hypothesis of a common source was ruled out.
3. The hypothesis of a noisy baseline was ruled out.
4. Very small delay of bilateral activity is a product of epileptic cell network dynamics.

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).

Fig. 2.

Schematic diagram of bilateral CA3 model **a**. Physiological basis of the model: two hippocampi connected by VHC. **b**. The bilateral CA3 model was built with two columns (in the middle) of neurons connecting through the hippocampal commissure. For either the left or right side, there were 10 cells in each row and 20 cells in each column ($N = 200$ for each side). Longitudinal propagation was only allowed in one direction (downward). To initiate the epileptic activity, pulse stimulation was applied to cell L1 (arrow) which has both ipsilateral and contralateral connections. Recording electrodes were assumed to be located at the center of each population. **c – f**: Four possible structures of the ipsilateral network were tested: “Random”, “In-Outward”, “All-Outward” and “All-Inward” connections, respectively (only upper part of left side of the model is shown, left and right side were symmetrical except for the “Random” ipsilateral connection). **g**: Compartmental cable model of a CA3 neuron. Early branching was modeled with two axons for simplicity.

Fig. 3.

Highly synchronized bilateral hippocampal activity *in vivo* **a:** With unilateral stimulation applied on left CA3 (triangle), the evoked potential recorded on the right CA3 verified the minimum interhemispheric propagation time was 6.7 ± 1.9 ms ($n = 10$). **b:** A 100 s example of 4-AP induced bilateral epileptic signal *in vivo*. **c:** Zoomed-in waveform shows the highly synchronized and continuous ictal activity. **d:** Further zoomed-in waveform and 50 Hz low-passed waveform show the delay between the onset sections of the two sides was very small. **e:** Plot of the maximum correlation coefficients between the two sides of the hippocampi, along with the time delays of maximal correlation demonstrated the strong bilateral synchrony. **f:** Cumulative probability histogram of the absolute value of bilateral delay ($n = 51$). Y-axis represents the cumulative probability for corresponding value of delay. Most delays were smaller than the physiological delay 6.7 ms, and the mean value was 3.66 ± 2.2 ms.

Fig. 4.

Highly synchronized bilateral hippocampal activity *in vitro* **a:** To measure the propagation time between left and right hippocampi, stimulation was applied in the middle of the VHC tract with simultaneous left and right CA3 recordings (images modified from Toprani and Durand, 2013). **b:** Pulse stimulation (triangle) in the middle of VHC axon tract yielded bilateral CA3 evoked responses, showing the complete interhemispheric propagation delay was 10.0 ± 2.7 ms. **c:** A 200 s example of 4-AP induced bilateral epileptic signal *in vitro*. **d:** Zoomed-in waveform shows the highly synchronized epileptic activity was made of multiple ictal events, interrupted by periods of interictal event. **e:** Further zoomed-in waveforms and 50 Hz low-passed waveform show the very small time delay. **f:** Plot of the maximum correlation coefficients between the two sides of the hippocampi and the time delays of maximal correlation verified the existence of the very small delay in this preparation. **g:** Cumulative probability histogram of the absolute value of bilateral delay ($n = 75$), 72% of which have a delay smaller than the physiological propagation delay 10.0 ± 2.7 ms. The mean value of delays was 7.9 ± 5.3 ms.

Fig. 5.

Very small delays cannot be explained by the noise hypothesis **a**: Seizure-like activity generated by the described computer model with "Random" ipsilateral connectivity. **b**: As a control experiment, all cells on the left side were simultaneously stimulated and evoked activity was recorded on the right side. This virtual control experiment revealed the time for evoked activity to travel through the VHC in a unilateral direction was 7 ms. **c**: Zoomed-in waveforms show a clear delay similar to the physiological propagation time. **d**: With additive noise applied, the bilateral delay was largely masked, yielding the appearance that seizure could start in two sides simultaneously. **e**: Sample signal of the *in vivo* experiments. The noise level (SNR) was measured to be 42 ± 5 dB ($n = 100$). **f**: Bilateral delay measured from the model is plotted as a function of the SNR, indicating that bilateral delay can be masked by noise when the SNR is less than 30 dB. Therefore given experimental noise level of 42 dB, the hypothesis that noise is masking cannot be supported.

Fig. 6.

Longitudinal propagation, as well as the transverse recurrent and feedforward connection play important roles in producing small delay **a**: A control experiment was conducted by unilaterally stimulating all cells on the left side and recording the evoked potential on the right side. This verified that the time for seizure to travel through VHC was 7 ms, as experimentally measured. **b**: Bilateral seizure activity generated by the “In-Outward” model. **c**: Zoomed-in epileptic events at different periods. The 7 ms delay was observed at the beginning of the event (left). Later, the two sides became gradually synchronized (middle), and the delay decreased to 1.5 ms at the end of the epileptic events (right). **d**: Histograms of delays calculated by cross-correlation between HCPs during different phases of simulated epileptic activity. Each bin in X-axis is 0.5 ms, and the Y-axis represents the number of HCPs. The delays between HCPs decreased from 7 ms (mean value) at the beginning to 5 ms at the half-way point, then to 1.5 ms at the end, showing all the cells on both sides were getting more synchronized over time. **e**: Delays of HCPs along the longitudinal direction throughout one burst. X-axis is the timeline. Different height of bar is used to facilitate

comparison. At the beginning, delay between L1 and R1 was equal to the physiological delay 7 ms, as the activity propagated longitudinally to further HCPs, the delay decreased gradually to as small as 0.5 ms (L61 and R61).

Fig. 7.

Effect of the ipsilateral network structure on the bilateral synchronization **a**: “Random” connections yielded non-periodical high frequency activity. **b**: “All outward” connections yielded periodical seizure like activity but with constant 8 ms delays. Zoomed-in signal is shown in the ellipse frame. **c**: “All-inward” connections yielded small amplitude spike-like activity with a delay of 5 ± 1.2 ms. **d**: Bilateral delay with different ratios of inward and outward connections. Small delays (< 2 ms) can be achieved with a ratio of 1/4 to 2, while minimum delay was achieved at a ratio of 1, i.e. a similar amount (ratio = 1) of inward and outward connections was required to generate the smallest bilateral delay.

Fig. 8.

Effect of the length of the VHC and size of the network on bilateral synchronization **a**: Alternating the length of the VHC within a range from 5 mm to 15 mm was tested. **b**: Bilateral delay with different VHC lengths is shown. The network was able to generate small delays (< 2 ms) only within a range of VHC length from 6 mm to 11 mm ($n = 5$). **c**: Size of the network was determined by numbers of cells in both longitudinal (m) and transverse (n) directions. **d**: A 3-Dimensional chart was constructed to show which combination of m and n could generate bilateral small delays, indicated by the height of the bar. Delays less than 2 ms were observed for combinations of m and n as follows: $6 < m < 200$ and $2 < n < 10$.

Table 1

CA3 neuron model specifications

C_m , F/cm ²	0.75	Mainen, et. al, 1995; Major et. al., 1994		
R_i , Ω /cm ²	255	Mainen, et. al, 1995; Major et. al., 1994		
C_e , S/m	0.49	Holsheimer, 1987		
	Diameter μ m	Length μ m	Segments	
Soma	20	30	1	Safronov et. al., 2000
Axon	1	D_n	20	Mainen, et. al, 1995
Dendrite	0.75	10	3	Mainen, et. al, 1995