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A Mechanism to Explain Zero-delay Bilateral Seizure Synchronization

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Abstract — Synchronization in bilateral CA3 regions via fimbria-fornix-hippocampal commissures system (FFHC) in rodent hippocampus has revealed that bilateral seizures can sometimes be synchronized with very small delays (< 1ms). This observed small time delay at the start of afterdischarges between the left and right CA3 regions is unexpected given the propagation time across the hemispheres (> 6ms). The possibility of a common source was first eliminated by in-vitro brain slices experiments. We then tested the hypothesis that, in the presence of noise, synchronization can take place before the seizure activity is sufficient large to be detected generating an apparent zero-delay between the two sides. This hypothesis was tested with computer simulation with a network of interconnected hippocampal neurons. These results provide an explanation for this aberrant simultaneous seizure detection and indicate the importance of noise in the interpretation of the timing of neuronal events.

I. Introduction

Epilepsy is the most common chronic neurological disease, affecting more than 50 million people worldwide. It is characterized by intermittent bursts of aberrant electrical activity in the brain resulting in seizure symptoms. Mesial temporal lobe epilepsy (MTLE), often signified by hippocampal sclerosis, is the most common and most medically refractory form of human epilepsy. A hallmark of this type of epilepsy is partial seizure with secondary generalization. One of the tracts which may be responsible for transmitting seizure activity bilaterally to the contralateral hippocampus is the fimbria-fornix-hippocampal commissures system (FFHC), as depicted.

The human dorsal hippocampal commissure (DHC) is a sizable tract and evidence for its functionality can be found in the literature. Furthermore, analysis of human EEG data shows that secondarily generalized MTLE occurs in the bilateral hippocampus before surrounding structures, suggesting there is an interconnecting pathway that is functional in seizure propagation [1, 2]. While the DHC is present in rats, the part of the commissural pathway that has been shown to be functional in in this species is the ventral hippocampal commissure (VHC). This has been demonstrated by Lima, et al. who showed that electrical activity in response to unilateral hippocampal stimulation of the CA3 region travels across the VHC to the contralateral hippocampus in rats [3]. An interesting observation of this study is the repeated zero-time delay between afterdischarges in the left and right hippocampi. This result is unexpected given the relatively long length of the FFHC axonal tract, which has been measured to be approximately 1.5 centimeters generating a propagation delay greater than 6ms between the two hemispheres.

The results reported by Lima, et al. were first replicated in an in-vivo rat model, where spontaneous ictal and interictal activity were induced by the potassium channel blocker, 4-aminopyridine (4-AP) and recorded extracellularly from the CA3 region of both hippocampi simultaneously (Fig. 3A). Though consistent with the literature, these observations are puzzling in that they do not conform with the model of spontaneous activity beginning in one hippocampus and travelling across the FFHC to the contralateral hippocampus since a minimum delay of 6ms would be expected

A possible explanation for this phenomenon is that there must be a common third structure that projects to both hippocampi and is equal distant from each but ruled out every possible candidate except for the VHC itself [3]. This explanation was first tested in-vitro by developing a novel preparation that preserves only the two hippocampi still connected by the ventral hippocampal commissure (VHC). A second explanation is that noise plays an important role in the synchrony measurement.

We propose an alternative explanation whereby spontaneous or triggered hyperactivity (or excitation) begins in one or a few neurons in either hippocampus and propagates across the commissures with the time delay one would expect given the anatomic properties of the cell and its environment. In the presence of noise however, the seizures are detected only when their respective amplitudes are greater than the noise level. When the seizures are detected they are already synchronized, thereby explaining the zero time delay. Computer simulation with two connected neuronal pools and additive noise is used to test the plausibility of such a mechanism.

II. Methods

Modeling of bilateral CA3 network

A bilateral CA3 network was modeled with MATLAB and neural modeling software NEURON [4-6]. Fig. 1 A shows the diagram of the network structure. The MATLAB program generates cell locations on each side randomly but within the area already specified. There are 2000 neurons on each side with 10% of which projecting to the contralateral

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side, the distance between populations is 15,000 um and the area is 200um x 400um x 200um [7, 8].

In NEURON simulation, all neurons are based on compartmental cable model whose parameters are shown in Table 1 [8]. We utilize a simplified cell model consisting of 2 dendrites with one excitatory synaptic input on each and two output axonal branches with excitatory synapses only. We model two axons since anatomically there is early branching. Only Hodgkin-Huxley channels are included in the model.

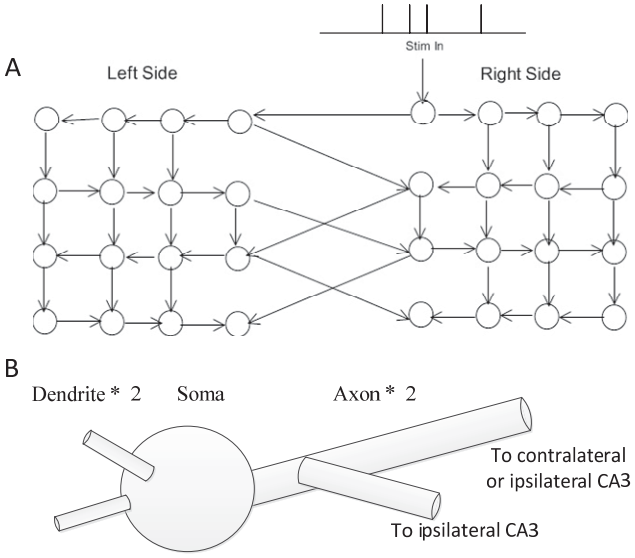

Fig. 1. A: Basic diagram of the bilateral CA3 network. To simulate epileptic activity, a random stimulation was applied unilaterally to the first cell which has both ipsilateral and contralateral projection. B: Compartmental cable model of CA3 neurons. Early branching was modeled with two axons.

Table I
CA3 neuron model specifications

C_m, F/cm^2	0.75
R_i, Ω/cm^2	255
C_e, S/m	0.49

Dimensions	Diamater μm	Length μm	Segments
Soma	20	30	1
Axon	1	D_n	20
Dendrite	0.75	10	1

The NEURON model specifications are shown. C_e stands for extracellular conductivity and D_n is the distance between two neurons.

Stimulus and noise modulation

To simulate the seizure activity, we applied random stimulation unilaterally to the first cell that has both ipsilateral and contralateral projection. Then the Gaussian noise is added to the seizure signal to simulate a realistic noisy environment. By adjusting the Signal-to-noise ratio (SNR), we calculate the delay between the seizure activities from the two sides with different noise levels.

The somas are considered to function as point sources for extracellular recording. The recording electrode is assumed to be at the center of each population. The equation

$V_e = i_m/(4\pi\sigma_e r)$, where

V_e = extracellular voltage at the recording electrode

i_m = the membrane current of a single soma

σ_e = the extracellular conductivity

r = the distance from the center of the soma (point source) to the recording electrode is used to calculate the contribution to the extracellular voltage from each individual soma at the location of the population's measuring electrode. To calculate the extracellular voltage recorded at the measuring electrodes over time, the contributions of the individual somas were summed for all points in time.

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 ip) and placed in a stereotaxic apparatus. Body temperature was maintained at 37 ºC with a heating pad. As shown in Fig. 2, several holes were drilled for placements of recording electrodes, return/reference screws and micro-syringes. Artificial cerebrospinal fluid (ACSF) was warmed to 37 ºC and applied to the exposed skull [9].

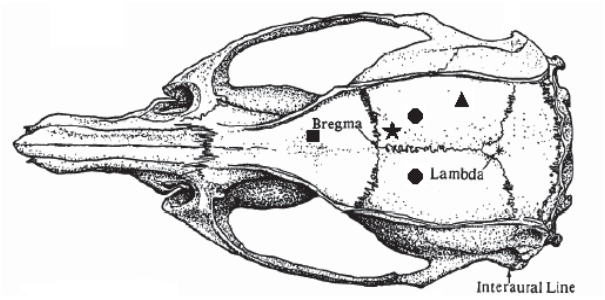

Fig. 2. Location of holes for placements of recording electrodes (two circles), return screw (pentagram), reference screw (square) and micro-syringe (triangle).

Solutions and drugs

Normal ACSF consisted of the following (in mM): 124 NaCl, 5 KCl, 1.25 NaH_2PO4, 2 $CaCl_2$, 1.5 $MgSO_4$, 26 $NaHCO_2$ and 2 g/L D-glucose. 25 mM 4-AP was injected to generate seizure, 1 ul for the first hour, and 0.5 ul for each subsequent hour.

Recording of the spontaneous epileptic activity

Recording probes (diameter 127 μm, Parylene-C, A-M Systems Inc., Carlsborg, WA) were positioned in both the left and right hippocampal CA3 regions (AP 3.0, ML ±3.0). Two stainless steel screws were fixed as shown in Fig. 2. Recorded signals were amplified 1000 times by Model 1700 4-channel amplifiers (A-M Systems Inc.) with filter frequency ranging from 1 Hz to 5 kHz [9]. Signals were then

sampled at a rate of 20 kHz with an ML795 PowerLab/16SP data acquisition system (ADInstruments Inc.) and stored into computer for off-line analysis.

III. Results

Zero time delay phenomenon during seizure activity in-vivo

Fig. 3 shows an example of two bilateral events from simultaneous recordings of 4-AP induced spontaneous activity from CA3 of bilateral hippocampi. The upper signal is from left CA3 and the bottom event is from right CA3. Analysis of the delay between many such events revealed frequent instances of such small delays between hemispheres.

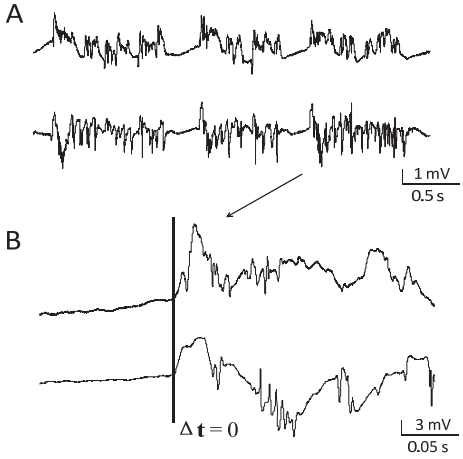

Fig. 3. A: Simultaneous recordings of in-vivo 4-AP induced spontaneous activity from CA3 of bilateral hippocampi. B: Zoomed-in waveform shows zero time delay. The propagation delay between the two CA3 regions as determined from electrical stimulation on one side and recording from the other is about 6ms (not shown)

Zero time delays are still observed in-vitro

A novel preparation that could preserve the two hippocampi still connected by the VHC was developed and seizure activity was observed with tissue thickness of around 750 um (See Fig. 4 A). Many synchronized events with a delay near 0ms were observed in this preparation (Fig. 4 B). Since there cannot be a third common source that could provide synchrony, this mechanism have to be abandoned.

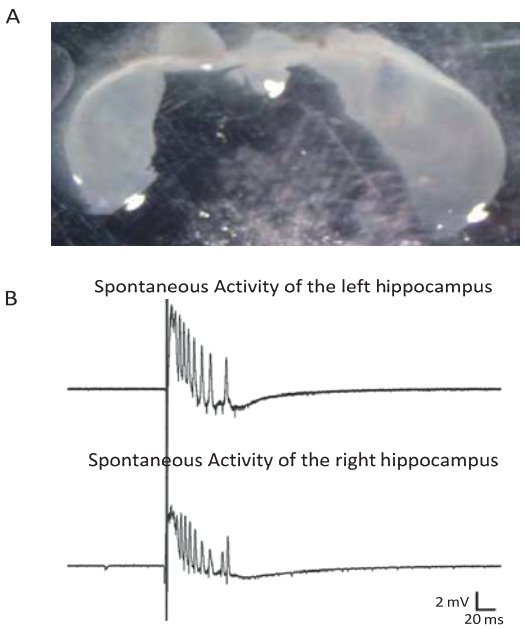

Fig. 4. A: Bilateral brain slice preparation: Both hippocampi and VHC are preserved. B: Simultaneous recordings of in-vitro 4-AP induced spontaneous activity from CA3 of bilateral hippocampi. Many such synchronized events were recorded thereby eliminating the possibility of a common extrinsic source generating the synchrony.

Synchronization in the presence of additive noise

Simulation results show that the Gaussian noise affects the signal delay between the two sides. With zero noise added, excitation in one hemisphere propagates to the other side with the expected delay (Fig. 5 A, B). In the presence of noise, synchronization can take place between the two hemispheres without any detectable delay. When the seizures finally emerge from the noise, the bilateral activity is already synchronized (Fig. 5 C) (With large noise, i.e. low SNR), very small delays are observed (Fig. 5 D). However, as the noise amplitude is decreased (increasing SNR), the seizure activity is more easily detectable yielding a noticeable delay well within the physiological range 6 to 12 ms (Fig. 5 D). This result is robust based on multiple simulations with various seed numbers for the random number generators (n = 100).

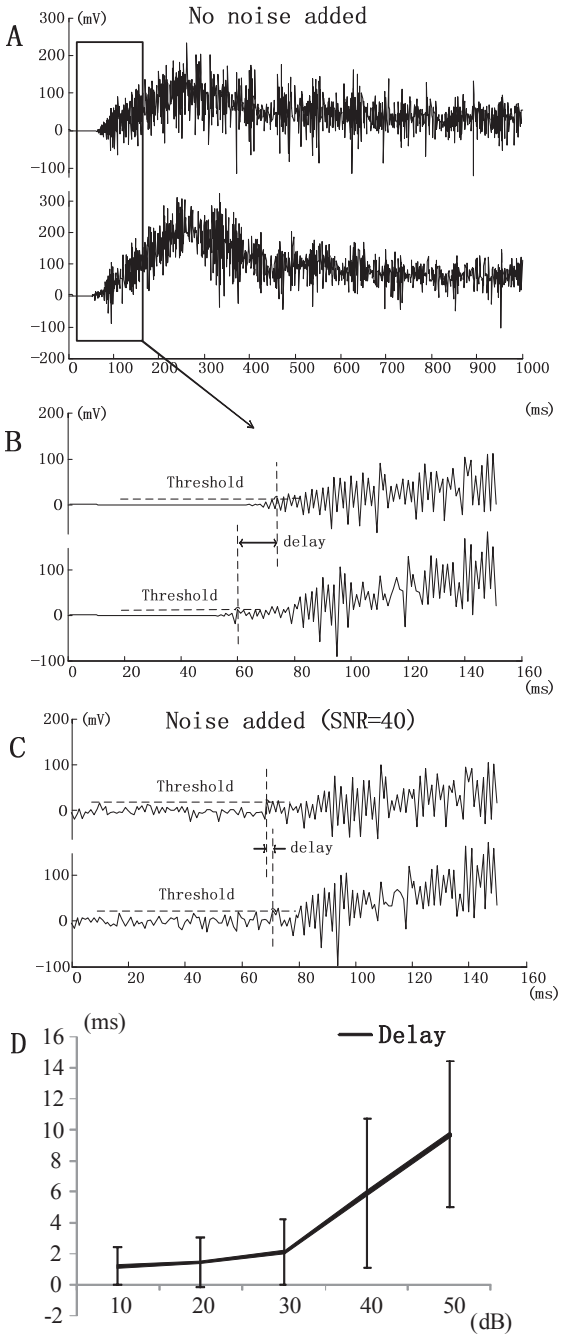

Fig. 5. A: Spontaneous epileptic activity generated by computer model. B: Zoomed-in waveform shows a clear delay which equals to the necessary propagative time from right CA3 region to the left via VHC. C: With noise added, the small delay event cannot be observed, yielding the appearance that the two sides start their seizure simultaneously. D: Quantitative analysis of measured delay under different noise levels as a function of SNR (dB). The results indicate that noise is responsible for the observed delay. (n = 100)

IV. DISCUSSION

The results of this simulation show that (1) Zero time delay phenomenon was replicated in 4-AP induced spontaneous epileptic rat model in-vitro, in-vivo and in-silico. (2) The in-vitro model eliminated the hypothesis that a common source could generate the synchronization (3) The computer model showed that additive noise plays an important role in yielding zero time delay phenomenon.

Epilepsy can be generated by multiple and varying foci which propagate the activity. Therefore the determination of the direction is necessary in studying the relationship between these foci. These analyses are done in the time domain with cross-correlation or similar method to analyze the phase relation of the simultaneously recorded waveforms. The leading position in time can also be influenced by other factors such as the presence of secondary epileptogenic mirror focus or varying speed of propagation. Here we show that the amplitude of the noise itself can influence the observed synchronicity of various events within the brain. These simulations were carried with additive noise, but noise plays a more important role since noise itself can participate in the synchronization of activity through coherence resonance.

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