Nonlinear Responses of Potentiated Hippocampal Slices to Mossy Fiber Stimulation

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Abstract

Phase-locked and chaotic responses of hippocampal CA3 slices to mossy fiber stimulation have been observed in a medium containing a $GABA_A$-receptor antagonist, penicillin. However, the network of the CA3 region was not normal, because inhibitory connections in CA3 were suppressed by the antagonist. In this paper, we investigated effects of the inhibitory connections on nonlinear responses of hippocampal CA3 slices in a normal medium. Long-term potentiation was induced at excitatory synapses in CA3 by tetanic mossy fiber stimulation, while preserving inhibitory connections. Potentiated hippocampal CA3 slices bathed in a normal medium caused phase-locked and irregular responses as well as the CA3 slices bathed in a medium containing penicillin. However, the slope of the boundary lines between different types of responses in the phase diagram was negative unlike CA3 slices bathed in a medium containing a $GABA_A$ antagonist.

Keywords: Hippocampus, CA3, LTP, Feedforward and feedback inhibition, Phase locking, Chaos

1. Introduction

It is supposed that the hippocampus has functions of memory and learning. The hippocampus has three major regions, the dentate gyrus, CA3, and CA1. CA3 is the most active region in the hippocampus because CA3 has many recurrent excitatory connections and CA3 pyramidal neurons tend to cause spontaneous activity.

CA3 of sagittal hippocampal slices causes spontaneous epileptiform bursts in an artificial cerebrospinal fluid (ACSF) containing penicillin because penicillin suppresses $GABA_A$ -receptor mediated inhibitions. Hayashi and Ishizuka have reported that phase-locked and chaotic responses of hippocampal CA3 slices to mossy fiber stimulation occur in a medium containing penicillin, and the responses have been summarized in a phase diagram [1]. However, the network of CA3 was, if anything, not normal, because inhibitory connections in CA3 were suppressed by the antagonist.

It has been found that in normal conditions, spontaneous epileptiform bursts occur in CA3 when long-term potentiation (LTP) is induced in CA3 by mossy fiber tetanic stimulation [2,3,4]. LTP was induced at both mossy fiber synapses and recurrent excitatory synapses in CA3 by tetanic mossy fiber stimulation. Nakashima and his coworkers have reported that epileptiform bursts is caused by LTP at recurrent excitatory synapses. In other words, epileptiform bursts do not require LTP at mossy fiber synapses [5].

In this paper, we investigated effect of the inhibitory connections on nonlinear responses of hippocampal CA3 slices in a normal medium. Potentiated hippocampal

CA3 slices bathed in a normal medium caused phase-locked and irregular responses to periodic mossy fiber stimulation as well as CA3 slices in a medium containing penicillin. However, the phase diagram in the normal medium is different from the phase diagram in the medium containing penicillin.

2. Materials and Methods

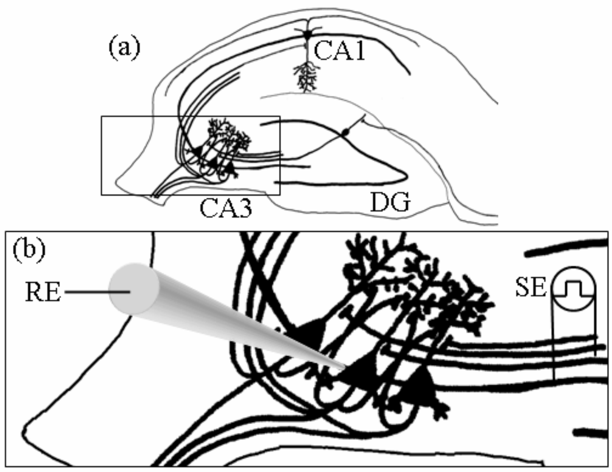

Fig.1 Location of the recording and stimulating electrodes. (a) Schematic picture of a sagittal slice of the rat hippocampus. (b) Stimulating bipolar electrode (SE) was placed on the mossy fiber tract in the hilar region to stimulate CA3 pyramidal cells. A glass microelectrode was placed on the stratum pyramidale (RE) to record the extracellular field potential.

Sagittal hippocampal slices, 400 μm thick, were obtained from male Wistar rats, 4-5 weeks of age (80-130 g). The hippocampus slices were placed on the liquid/gas interface chamber maintained at 34 ± 0.5 °C. ACSF bubbled with 95% O_2 and 5% CO_2 was perfused. The atmosphere around slices was warmed to keep 34 ± 0.5 °C and saturated with 95% O_2, 5% CO_2, and steam. The composition of ACSF was 123 NaCl, 1.3 $MgCl_2$, 5 KCl, 2 $CaCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 glucose (in mM).

Field potential was recorded by a glass microelectrode placed on the stratum pyramidale in CA3, as shown in Fig. 1(b)(RE). The tip diameter of the microelectrode was 20-30 μm. The mossy fiber tract in the hilar region was stimulated by a bipolar electrode, as shown in Fig. 1(b)(SE). The bipolar electrode was made of urethane-coated stainless wire (50 μm in diameter). The distance between uninsulated tips of the bipolar electrode was approximately 0.5 mm. Tetanic stimulation that consisted of three-pulse bursts (100 Hz) repeated 34 times at intervals of 100 ms was applied to the mossy fiber tract to induce LTP and cause spontaneous epileptiform bursts in CA3. The amplitude of stimulus current pulses I was normalized by the threshold current I_{th} that initiated an epileptiform burst when the threshold current was applied 3 s after a spontaneous epileptiform burst.

3. Results

Fig. 2(a) shows the phase diagram of field potential responses of CA3 to periodic mossy fiber stimulation. Slices were bathed in a normal medium, and LTP was induced by mossy fiber tetanic stimulation before responses to periodic stimulation were recorded. Control parameters are the normalized current I/I_{th} and the frequency of mossy fiber stimulation f.

1:1, 1:2, and 1:3 phase-lockings occur in the regions marked with open symbols, ○, Δ, and □, respectively. Complex phase-lockings and irregular responses occur in the regions marked with closed symbols, ● and x, respectively.

The slope of the boundary lines between different types of response in the phase diagram is positive in the range of the normalized stimulus intensity below 1.5. However, the slope of the boundary lines is negative in the range of the stimulus intensity above 1.5. When I/I_{th} = 2.0, responses are irregular, and the burst firing probability is markedly low. Fig. 2(b) shows the phase diagram that has been obtained by Hayashi and Ishizuka [1] using hippocampal slices bathed in a medium containing $GABA_A$ antagonist, penicillin. Therefore, inhibitory circuits in CA3 were suppressed. The slope of the boundary lines between different types of responses is always positive, and CA3 slices well respond to mossy fiber stimulation even at a high intensity of periodic stimulation.

The difference between the phase diagrams would result from afferent and/or recurrent inhibition in CA3. Intense stimulation of moss fibers would increase effect of the inhibition, and it would become less easy for CA3 to respond.

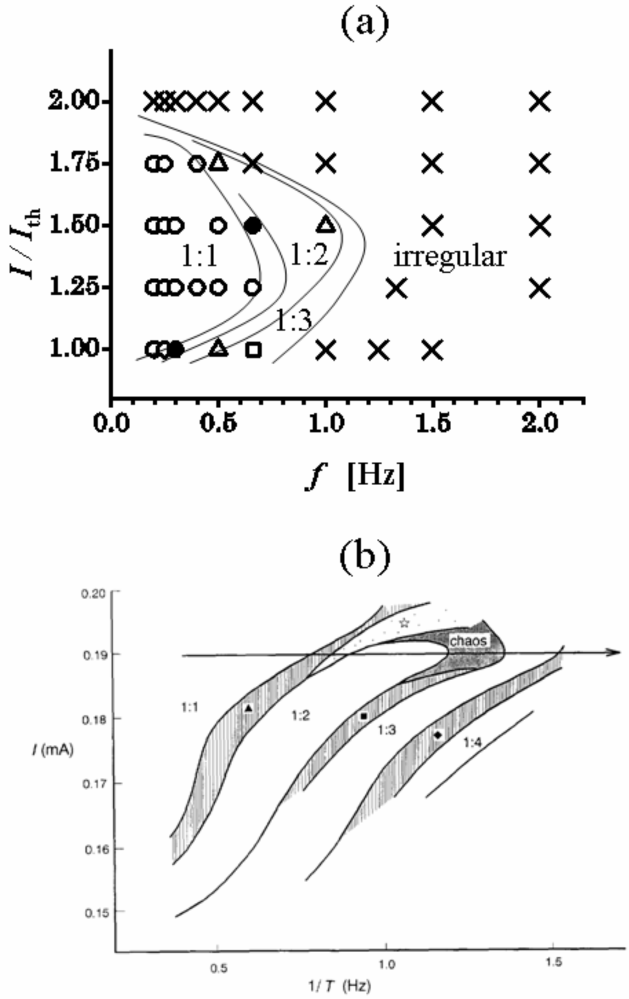

Fig.2 Phase diagrams of field potential responses of CA3 to periodic mossy fiber stimulation. (a) Phase diagram. Slices were bathed in a normal medium, and LTP was induced by tetanic mossy fiber stimulation. Abscissa is the frequency of periodic stimulation of mossy fibers. Ordinate is the stimulus intensity; the amplitude of stimulus current pulses I was normalized by the threshold current I_{th}. Complex responses that seem to be a mixture of different phase-locked responses occur in the region with symbol ●. (b) Phase diagram. Slices were bathed in a medium containing $GABA_A$ antagonist, penicillin [Hayashi and Ishizuka, 1995]. Tetanic stimulation was not applied to mossy fibers. $1/T$ is the frequency of mossy fiber stimulation. I is the stimulus current.

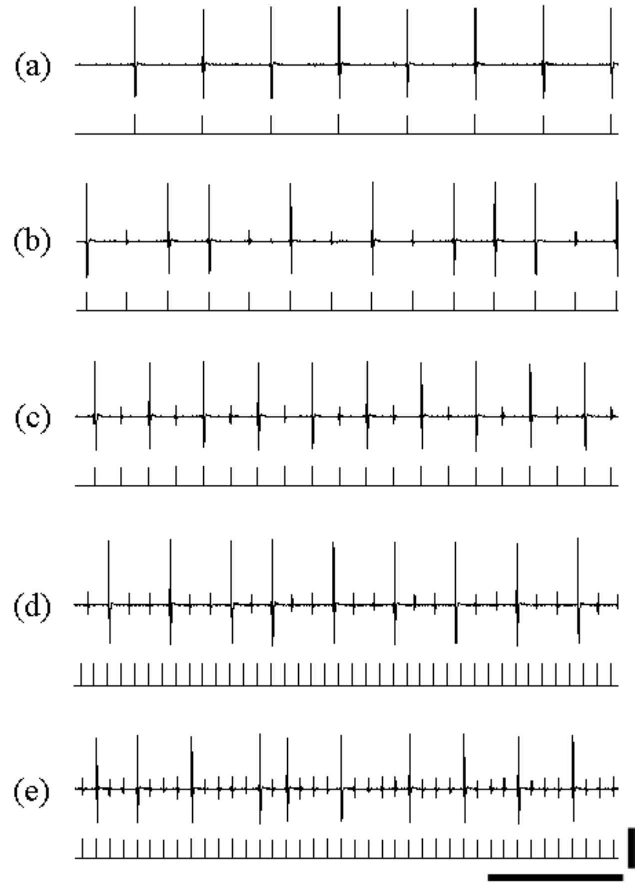

Fig.3 Time series of epileptiform bursts in the CA3 region caused by periodic mossy fibers stimulation. The upper and lower traces in each panel are 40 s segments of the field potential and stimulus current pulses, respectively. The amplitude and duration of the current pulses are 140 μA (I/I_{th} = 1.0) and 100 μs, respectively. (a) 1:1 phase-locking. The frequency of periodic stimulation is 0.25 Hz (Intervals are 4.0 s). (b) Complex response observed in the region between 1:1 and 1:2 phase lockings. Response is a mixture of 1:1 and 1:2 phase lockings. The stimulus frequency is 0.33 Hz (3.0 s intervals). (c) 1:2 phase locking. The stimulus frequency is 0.5 Hz (Intervals are 2.0 s). (d) 1:3 phase locking. The stimulus frequency is 0.67 Hz (Intervals are 1.5 s). (e) Irregular response. Response is the mixture of several kinds of phase lockings. The stimulus frequency is 1.0 Hz (Intervals are 1.0 s). The vertical and horizontal bars indicate 1.0 mV and 10 s respectively.

Fig. 3 shows the time series of field potential responses to periodic mossy fiber stimulation. LTP was induced in CA3 by tetanic mossy fiber stimulation in a normal medium. The upper and lower traces in each panel are 40 s segments of the field potential and stimulus current pulses, respectively. Fig. 3(a), (c), and (d) show 1:1, 1:2, and 1:3 phase lockings respectively. Fig. 3(b) shows the complex response observed in the region marked with ●. The response is a mixture of 1:1 and 1:2 phase-lockings. Fig. 3(e) shows a more complex response that is a mixture of several kinds of phase-lockings.

4. Discussion

We investigated effects of the inhibitory connections on nonlinear responses of hippocampal CA3 slices in a normal medium. Long-term potentiation was induced at excitatory synapses in CA3 by tetanic mossy fiber stimulation, while preserving inhibitory connections. Spontaneous bursting discharges were also caused after LTP was induced as well as in a medium containing a $GABA_A$ antagonist.

Potentiated hippocampal CA3 slices bathed in a normal medium caused phase-locked and irregular responses to periodic mossy fiber stimulation as well as the CA3 slices in the medium containing penicillin. However, unlike the CA3 slices in a medium containing a $GABA_A$ antagonist, the slope of the boundary lines between different types of responses is negative in the range of the normalized stimulus intensity above 1.5 in a normal medium. In other word, the frequency region of each type of response became narrow with increase in the stimulus intensity.

The difference between the phase diagrams would result from inhibitory connections in CA3. Two kinds of inhibitory connections exist in CA3: feedforward inhibition at afferent synapses and feedback inhibition at recurrent synapses. Antidromic stimulation of Schaffer collaterals can activate only feedback inhibition, besides recurrent excitation. Our preliminary data show that the potentiated hippocampal CA3 slices causes phase-locked and irregular responses to periodic Schaffer collateral stimulation, and the slope of the boundary lines in the phase diagram is positive (data not shown) like the phase diagram of the responses of CA3 slices in the medium containing penicillin.

This indicates that recurrent inhibition does not have much effect on the responses to mossy fiber stimulation. In other words, this suggests that the negative slope of the boundary lines in the phase diagram results from the feedforward inhibition at afferent synapses that is activated by mossy fiber stimulation.

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