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Brain waves and "Interactome"

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Abstract- Hippocampus has a potential to induce many neuronal rhythms, that is, theta rhythm, beta rhythm, gamma rhythm and epileptic discharges. In the neurons in the hippocampal network, there is the molecular network called "interactome". The interactome includes protein-protein network, protein-gene network etc. In the present paper, we paid attention to the interactome which is related to the synaptic plasticity. We studied the effect of the interactome on epileptic discharges which were induced by the application of bicuculline and the carbachol-induced beta oscillation. Our results suggested that the interactome plays an important role in the induction of the neuronal rhythms.

1. Introduction

There are some brain rhythms, the electroencephalographic activities, observed in the brain. It is thought that the rhythms take part in the information processing of the brain. The rhythms come from the neuronal network in the brain. The neuronal network has synaptic plasticity. It is called long-term potentiation (LTP) and long-term depression (LTD).

In the induction of LTP, the depolarization of the post synaptic cells triggers the process. The depolarization induces the opening of N-methyl D-aspartate (NMDA) receptors/Ca^{2+} channel, and extracellular Ca^{2+} flows into the cell. Intracellular Ca^{2+} activates Ca^{2+}-dependent protein kinase such as Ca^{2+}-dependent protein kinase II (CaMKII) and LTP is induced.

In the neuronal cells, the intracellular molecules, such as Ca^{2+}, calmodulin, CaMKII and so on, interact each other and form the molecular network called "interactome" [1]. We studied the relationship between the "interactome" and the rhythmic phenomena induced by the neuronal network. We will introduce the two results and the developing brain simulator project.

2. Bicuculline-induced epileptic discharges

Guinea-pig hippocampal slices which were 500 μm thick were used in this experiment (Fig. 1). Hippocampal network has the excitatory and inhibitory neurons. When the inhibitory synaptic transmission is suppressed, the epileptic discharges are induced. Actually, when bicuculline (BIC), a GABAa receptor antagonist, was applied to hippocampal slices, epileptic discharges are induced (Fig. 2). Bicuculline-induced epileptic discharges (BIED) appeared first at ten minutes after BIC application. At that time, the BIED frequencies were transiently high. Then they decreased to the steady state for 10 minutes. BIED could be also observed even from the isolated CA3 mini slices. Hence, the generator of BIED should be in CA3 region. AP5, an NMDA receptor antagonist, decreased the BIED frequency but did not suppress its generation. KN-93, a CaMKII inhibitor, significantly decreased the frequency of BIED, while KN-92, a negative control of KN-93, did not (Fig. 3). Another CaMKII inhibitor, KN-62, also decreased the frequency. NMDA receptor and CaMKII play important roles in the induction of LTP in hippocampus. Thus LTP phenomena can increase the frequency of BIED. Especially we hypothesized that LTP at excitatory synapse would be involved. We applied CNQX, an AMPA receptor antagonist, to BIED and it decreased the BIED frequency. At the time excitatory synapse was also suppressed, and therefore, synaptic transmission at excitatory synapse can modulate the frequency of BIED. Taken together with the results of the isolated slices, the excitatory transmission in the hippocampal neural network of hippocampal CA3 region is related to the induction of BIED.

It is clarified that the molecular network in neurons affects on the rhythmic phenomena of the hippocampal epileptic discharges. In the details, feedback inhibition is effective in the CA3 hippocampal network before BIC application, and the pyramidal cells in the network are not relatively noisy. After BIC is applied, the inhibitory postsynaptic potential is suppressed, the pyramidal network becomes noisy, and among the pyramidal neurons the pacemaker neurons to pace BIED emerge. The pyramidal network in which the synaptic connections are strengthened via both the NMDA receptor- and the CaMKII-dependent processes receives the output of pacemaker neurons, and it can induce BIED.

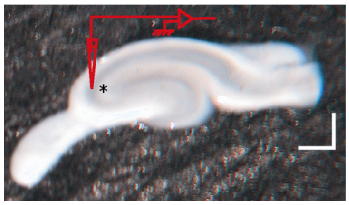

Fig. 1. Schematic picture of the transverse slice of guinea-pig hippocampus and the placements of recording electrode (red) and CaMKII inhibitors application electrode. An extracellular recording

electrode (red electrode) was placed in the *stratum pyramidale* of CA3. Black asterisk indicates the application site of CaMKII inhibitors and KN-92.

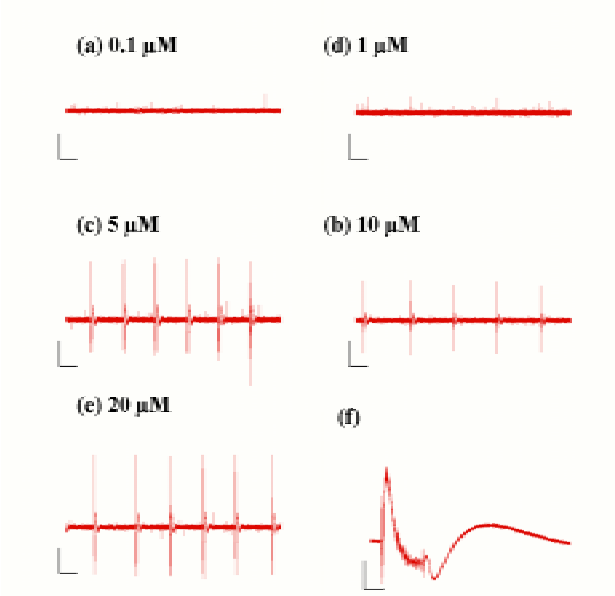

Fig. 2. The epileptic discharges induced by different concentrations of bicuculline (BIC) in the CA3 hippocampal slices. Typical examples of a train of epileptic discharges at different concentrations of BIC are shown ((a)-(e)). Above 5μM BIC could induce the discharges. (f) is a typical example of a epileptic discharge induced by 5 μM BIC. Note that the time is expanded in (f). Scale bars: (a)-(e); 5 sec, 0.5 mV, (f); 100 msec, 5 mV.

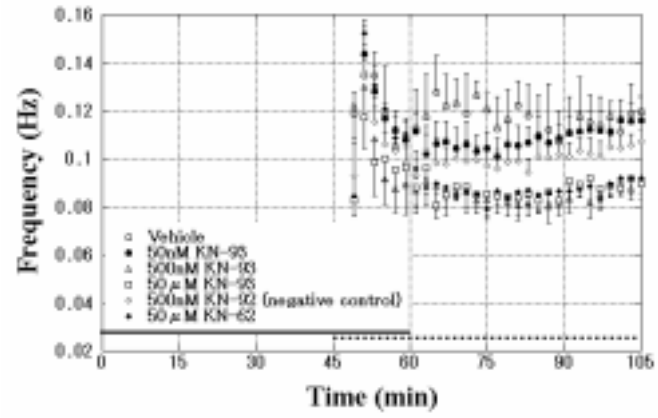

Fig. 3. Effects of CaMKII antagonists on the epileptic discharges. The solid bar indicates the administration period of the drugs. Dashed line indicates the application period of 20μM BIC. Five hundred nM and 50 μM KN-93 decreased the frequency, while KN-92 couldn't. KN-62 also decreased it, too.

3. Carbachol-induced beta oscillation

Rat hippocampal slices with 4-500 μm thick can induce beta oscillation when carbachol, a cholinergic agent, is applied to the slices. With the application of 30 μM carbachol, the rhythmical activity whose frequency was 12-20 Hz was recorded from CA3 region of the slices (Fig. 4). The frequency was 17.29+- 0.37 (mean +-s.e.m.) Hz and it is in beta range. Thus we called the oscillation beta oscillation. When you added bicuculline to the oscillation, the frequency of beta oscillation decreased. With the application of 30μM carbachol and 5μM bicuculline, the frequency was 12.31 +- 0.36 Hz. Under this condition, the schaffer collaterals were stimulated with the theta-burst stimulation (TBS) by tungsten bipolar electrode. From then LTP in CA3 was immediately induced just after TBS. The frequency of beta oscillation was not changed then. Thirty minutes after the stimulation, the frequency of the activity gradually decreased (Fig. 5). When LTP was suppressed by AP5, the frequency did not change. These results suggest that the LTP induced by TBS decreases in the frequency of carbachol-induced beta oscillation [3]. As LTP is induced by the activation of interactome, these results also suggested that the interactome can affect the electrical neuronal rhythms.

Alternatively, when AP5 was applied from before the application of carbachol, beta oscillation could not be induced. The beta oscillation also failed to be induced, when KN-93 was pre-applied before the application of carbachol. Theses results suggest that the interactome which relates to the synaptic plasticity plays an important role in the induction of carbachol-induced beta oscillation as well as the frequency modulation of the oscillation.

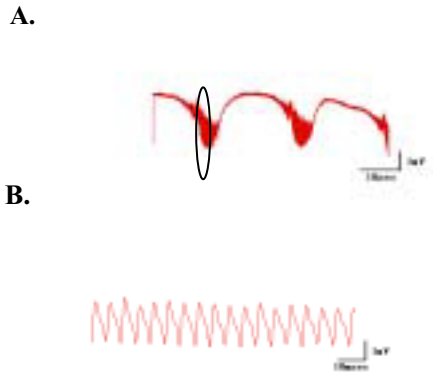

Fig. 4. Carbachol-induced beta oscillation observed in rat hippocampal slices. Oscillations occurred intermittently with the period of 20-30sec. B is expanded from the circle in A. Calibration bars; horizontal, 10sec (A) and 100msec (B); vertical, 1mV.

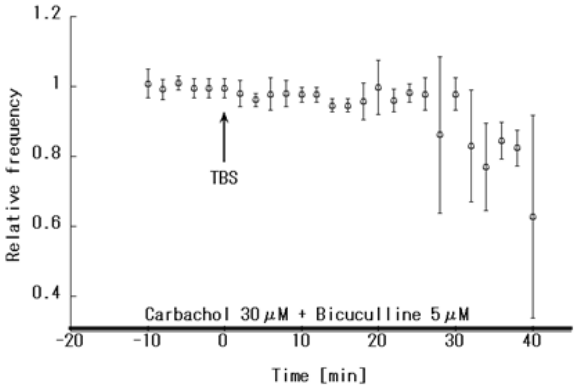

Fig. 5. Effects of TBS on beta oscillation.
The averaged relative frequency of beta oscillation decreased about 30 minutes after TBS (n=4).

5. Brain simulator "HIPPO-STATION" [4]

The rhythmical activity of neurons in hippocampus is linked to the intracellular interactome. To study the relationship in detail, it is necessary to have a simulator which can simulate neuronal electrical phenomena and intracellular biochemical network "interactome". Hence, we are developing a brain simulator called "HIPPO-STATION" (Fig. 6). HIPPO-STATION is a graphical user interface (GUI)-based simulator coded by the Java language. It contains neuroinformatics and bioinformatics simulators. In neuroinformatics simulations, Hodgkin-Huxley type equations are generally calculated and our "HIPPO-STATION" has adopted the equations. Using the simulator, you can easily knockout and knock-in several kinds of channels in the neurons. In bioinformatics simulator, HIPPO-STATION adopts the mass action reaction equations, Michaelis-Menten equation, Hill-equation. If you use the bioinformatics simulator, you can do the experiment of knock-out and knock-in of molecules and learn the role of the molecules in the interactome. The system consists of an Oracle database server, client machines and the network. The clients access the server via a network. The database server includes parameters which are used in the neuroinformatics and bioinformatics simulation.

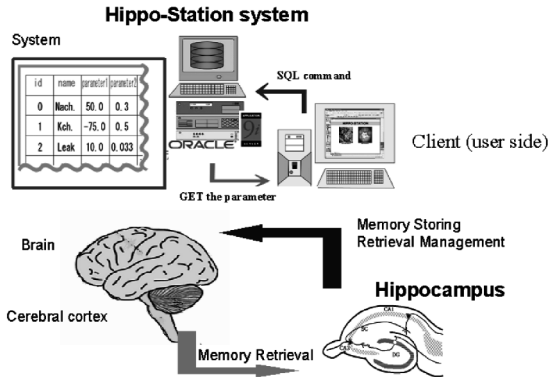

Fig. 6. HIPPO-STATION system. The system consists of an Oracle 9i database server and simulator clients. The database contains the parameters of model simulations and the images of neurons and brains of animals. Several kinds of model neurons and the intracellular interactome are implemented in the system. First, the client accesses the server in order to obtain simulation parameters. Once the client gets the parameters, the system can simulate the model by itself. HIPPO-STATION is programmed by java language, because the system is planned to be web-accessible through internet.

Acknowledgments

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