【Grant-in-Aid for Scientific Research (S)】

Broad Section G

Title of Project： From the structure-function relationship of dendritic spines to synaptic mechanobiology

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Keyword：Learning, Memory, Synapses, Cell motility. Mechanobiology

【Purpose and Background of the Research】

The depth penetration of the two-photon microscope allowed us to examine the structural plasticity of dendritic spines in 2000s. We have further developed the two-photon uncaging technique which allowed us to stimulate single submicron dendritic spine by glutamate, and found the spine sizes themselves changes and they are tightly related to the learning *in vivo*. In the present proposal, we will extend our investigations into the presynaptic terminals to test whether spine enlargement has direct mechanical effects on the presynaptic terminals. We first study rapid mechanical effects of spine enlargement, and then long-term effects by using the optical probes. In this manner, we developed the new field where short and long term mechanical action to presynatpic terminals are investigated.

【Research Methods】

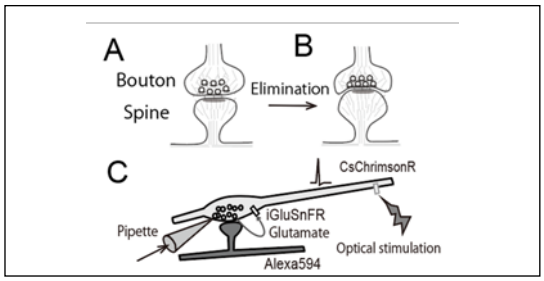

Figure 1 Mechanical interactions of synapses

Learning stimuli have been known to induce spine enlargements, which inevitably push the presynaptic terminal making synaptic contact with the spine. This type of effects has anticipated but never been experimentally examined due to the difficulty of the experiments. We are now challenging the issue, by using the two-photon uncaging, glutamate sensor protein, optogenetics, SNARE/FRET probe to quantify the engine of exocytosis, Q-dot coated glass pipettes, and founds that the pushing caused assembly of SNAREs, and facilitate evoked exocytosis. A similar effect can be seen with osmottic pressure, and learning-stimuli induced spine enlargement. Thus, axonal boutons have the pressure sensation and transduction (PREST) mechanisms with which synapses interact mechanically in addition to well-known chemical and electrical transmissions. Interestingly, the one minute pushing caused more than 20 min faciliatoty effects, and may act as working memory.

We have investigated the relationship between learning and behavior using the nucleus accumpens, and found that direct pathway mediate generalizing reward learning and the indirect pathway the discrimination learning, and these conditioned learning depend on the dopamine mediated spine enlargements in respective neurons using slice and in vivo preparation.

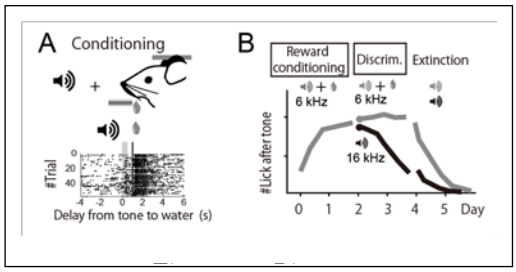

Figure 2 Generalization/discrimination in mice.

【Expected Research Achievements and Scientific Significance】

We will fast publish our amazing PREST mehanisms by completeing all the additonal experiments asked by reviewers. Then, we study the cellular and molecular bases of PREST using STED superresolution microspy to find either pharmacological or genetic intervention of PREST effects to identify the working memory role in vivo. We also obtain a new line of evidence for the synaptic bases of the conditioned learning.

【Publications Relevant to the Project】

- Takahashi, N., Sawada, W., Noguchi, J., Watanabe, S., Ucar, H., Hayashi-Takagi, A., Yagishita, S., Ohno, M., Tokumaru, H. & Kasai, H. (2015). Two-photon fluorescence lifetime imaging of primed SNARE complexes in presynaptic terminals and □ cells. *Nature Communications* 6:8531.
- Iino, Y., Sawada, T., Yamaguchi, Tajiri, M., K., Ishii, S., Kasai, H.* & Yagishita, S.* (2020) Dopamine D2 receptors in discrimination learning and spine enlargement. *Nature* 579: 555-560.

【Term of Project】 FY2020- 2024

【Budget Allocation】 150,700 Thousand Yen

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