1P226 スパイク列に見られる履歴と神経細胞の特性との関係

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Relation between spiking history and properties of neurons

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Spike train is one of the main information carriers in nervous system. It is not yet clarified which statistic of the spike train is the carrier in nervous system. It is important to understand the factors simultaneously affect the spiking activity of individual neurons to understand the information carrier or to realize neuro-motor prosthesis. Recent analysis of spike trains using a point process framework shows action potential generation is affected by spiking history (Truccolo et al., J. Neorphysiol., 2004). One can assume two kinds of source of the spiking history dependence. One possible source is from the network structures of nervous system. The other one is from action potential generation mechanism, i.e. individual neurons' activities depend on the past history. Thus, it is important to study on the mechanism of spiking history in individual neurons. In our study, by using a neuron model, we analyze the mechanism of the history and discuss how it affects the information carrier in nervous system.

1P227 アガロースマイクロ構造内に培養した神経1細胞の長期活動計測

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Long-term recordings of cultured single neuron in agarose microstructure.

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We previously reported a single-cell-based on-chip multi-electrode array (MEA) cell cultivation system combined with an agarose microchambers (AMCs). It is possible to record the firing at single cell level for long term and topographically control the cell position and connections. This time, we reported the spontaneous firing of cultured single neuron in an agarose micro chamber for long term. We succeeded in culture of Rat hippocampal neuron (E18) over 1 month by using glia-cells-derived diffusible factors, and in recordings of spontaneous firings before and after tetanic stimulation for several days. For tetanic stimulation, 10 trains of 10 pulses of the bipolar pulse (100 μs at + 15μA, followed by 100 μs at -15μA) and duration at 50 Hz were applied 10 times at 5-s intervals. As a result, we detected rhythmic burst firings of single neuron and effects of tetanic stimulation. In the meeting we will present the results in detail and will discuss the potential of our method.

1P228 一細胞レベルにおける学習・記憶形成に関わる分子機構解析

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Single Cell Analysis for Memory Formation in Molluscan Central Nervous System

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Taking advantages of molluscan nervous system, its simplicity and large neurons, we here discuss the neuronal and molecular substrates for memory formation of associative learning at a single cell level. Cyclic AMP-responsive element binding protein (CREB) is universally accepted to be necessary for specific transcription in long-term memory formation. In the key neuron of associative learning in the pond snail *Lymnaea stagnalis*, we first showed the inhibition of CREB function blocked the expression of cAMP-induced synaptic plasticity at a single cell level. We then characterized the CREB genes in *Lymnaea* central nervous system (CNS), including transcriptional activator CREB1 and repressor CREB2. Interestingly, CREB1 transcripts included the repressor isoforms as well as the activator ones. The interaction between the activator and the repressor CREB1 protein was demonstrated in co-transfected HeLa cells using dual color fluorescence cross-correlation spectroscopy (FCCS). Real-time RT-PCR experiments showed the transcriptional repressor CREB1 isoforms and CREB2 were constitutively expressed at large amount, as well as activator CREB1. The copy number of CREB1 and CREB2 mRNAs was changed according to training paradigm and their behavior, at the CNS and a single cell level. These results suggest that the transcriptional ability of CREB is regulated by altering the ratio between the transcriptional activator and repressor proteins, and to the change of synaptic plasticity in the key neuron for associative learning.

1P229 自然淘汰型進化リアクターを使ったプロモータの進化実験

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Evolution of promoter in a natural selection-type evolution reactor

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Objectives of this study is to get the optimal TT7 promoter sequence at 50°C by *in vitro* evolution using a "natural selection"-type evolution reactor based on RNA-Z isothermal amplification using HIV-RT and TT7 RNA polymerase in 1.4 M trehalose, and to study the evolution process as an adaptive walk on the sequence space. In the previous study, we observed a context-dependent selection through a neutral path. When we expressed the process of evolution by information quantity, we found a transient divergence in the process of final convergence. When we measured the selection coefficient by competition experiments, we observed that the selection coefficient was nearly constant during sequence divergence phase. In this study we started from another template library, which had a random region and a stable context, that is, the final context in the previous study. Evolution process of the new template is fast and single stepped to reach the strongest promoter identical to the previous one. When we started from another template having an A-start T7φ2.5 promoter context(not so stable), we observed also a context-dependent selection to reach the G-start promoter. We made the library with the stable A-start T7φ2.5 promoter context and carried out similar experiments to confirm such phenomenon.