“E-Neuron Project: Kinetic simulation of LTD using E-CELL system”

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Abstract

Cerebellar Purkinje cells exhibit a specific type of synaptic plasticity, called long-term depression (LTD). A number of cellular signal transduction pathways are known to induce LTD, but the entire mechanism still remains to be obscure. We constructed a computational model of Purkinje cell, including almost well known and novel signal transduction pathways. We simulated this model using E-CELL system, which is simulation platform for the modeling of cell at a molecular level, and can simulate metabolic interactions but can also contain gene expressions. We expressed distinctive features of LTD and predicted some viewpoints.

Summary

Postsynaptic reactions make synaptic plasticity. It is especially well known as cerebellar long-term depression (LTD) and hippocampal long-term potentiation (LTP). Both of them are considered to hold a relation to molecular and cellular basis of learning and memory. But the systematic relationship between synaptic plasticity and the currently known pathway remain obscure. There are also unknown pathways that may exist. Meaning, computational kinetic simulation is an optimal tool to revealing the mechanisms of synaptic plasticity.

To make a computational model and simulate cerebellar Purkinje cells, we followed the kinetic simulation done by Kuroda using GENESIS/kinetikit (Kuroda et,al, 2001).
It is well known that long time stable phosphorylation of AMPA receptor induces LTD, since it induces the reduction of the amount of AMPA receptor on the surface.
Therefore, in their simulations, the final output of the simulation is the phosphorylation of AMPA receptor. In their model the phosphorylation of AMPA receptor consist of initial phase and intermediate phase.
Because it has been shown that gene expressions and protein synthesis are required

for the late phase of cerebellar LTD, but their model incorporate only biochemical parameters of post-transactional biochemical reactions.
The pathway of the simulation structure, the internal of neurons being complicated as it is, seemed somewhat obscure when considering the input signal and the output to AMPA receptor's phosphorylation. However, there are some well-known pathways, of which concerns phosphorylation of AMPA receptor.

In the conventional model, if stimulated only by the climbing fiber (CF), phosphorylation of the AMPA receptor underlying the initial phase of Cerebellar LTD could not be observed like so in the experiment. This result indicates the possibility that there is another mechanism existing in addition to the phosphorylation of AMPA receptors underlying the initial phase of cerebellar LTD. So we brought focus to this point, and came up with the viewpoint of PKG having a permissive role to phosphorylation of AMPA receptors at the initial phase. We think that there is a cascade, which derives from the stimulation of CF to the activation of PKG, and it is taken into consideration to essential substrate at the initial phase of phospholylated AMPA receptors only by the stimulation of CF.

In this work, we improved the conventional simulation model. And we simulate this model using E-CELL system, simulation environment for the modeling of cells at a molecular level, which, can simulate not only metabolize interactions but can also contain gene expressions. In the experiment part, we have skills that enable to inactivate a protein immediately using laser beam called microscale chromophore-assisted laser inactivation (micro-CALI). This experimental part enables us to verify the simulation result and provide us basis of molecular biology of the cell.

At this time, we construct the kinetic simulation model of cerebellum, included PKG as a phosphatase of the AMPA receptor and production of NO derive from internal Ca2+. First, we simulated the situation of conjunctive stimulation by PF and CF. Then compared the situation stimulated by PF or CF alone. And we simulated from another viewpoint. It is useful to inactivate one protein to find out how it works. In this simulation, several proteins be inactivated and observed what role it has.

In our simulation of cerebellar LTD, we succeeded in the reproduction of phosphorylation of AMPA receptor stimulated by CF only. Influx of the Ca2+ induces the activation of CaM. Activated CaM activate nNOS, and it made Arg into NO and Cit. Leading, NO / cGMP pathway activates the PKG. Activated PKG phosphorylate the AMPA receptors. This cascade made CF stimulation possible to phosphorylate AMPA receptor. So, our model makes it possible to reproduce the phosphrylation of the AMPA

receptors stimulated not only by PF and CF but also by CF only. Of course this model has bistable of phosphorylate the AMPA receptor, it makes synaptic plasticity.

For discussions, it is quite well known that gene expressions required the late phase of phosphorylation of AMPA receptors. Therefore, it is important to include gene expressions to reproduce late phase of cerebellar LTD model.

Recent reports have been shown that Ca2+ has a permissive role for signal transduction pathway. Ca2+ is harmful for the cell, but it has quite an important role at signal transduction. At hippocampus, Ca2+ may regulate where synaptic plasticity has been occurred, LTD or LTP. But precise Ca2+ role and reactions remain obscure. Our project, we tried to reveal the mechanism of hippocampal LTD and LTP. This simulation model would represent the whole system of hippocampal synaptic plasticity.

In the near future we will try CALI on E-CELL and predict unknown pathways and signal molecule using our method.