【Grant-in-Aid for Specially Promoted Research】

Biological Sciences

Title of Project : Elucidation of the roles of synaptic retrograde signaling in functional neural circuit formation during postnatal development

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Research Area : Neurophysiology/general neuroscience
Keyword : Molecular and cellular neuroscience

【Purpose and Background of the Research】

Neuronal connections are initially redundant, but they are refined and become functionally mature during postnatal development. This process is known as 'synapse elimination' and is widely accepted as a crucial step to refine redundant neural circuits into functionally mature versions. The climbing fiber (CF) to Purkinje cell (PC) synapse in the cerebellum provides an excellent model to study the process of synapse elimination.

Previous studies indicate that activity of postsynaptic PCs is crucial for this process. Hence, 'retrograde' signaling mechanisms must exist which transmit signals from postsynaptic PCs to presynaptic CFs. However, little is known about such mechanisms.

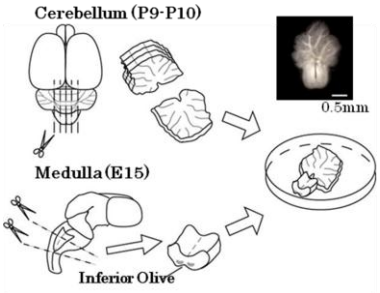

Fig. 1 Olivo-cerebellar coculture preparation

We have recently developed an organotypic coculture preparation that consists of a cerebellar slice and a medullary explant containing the inferior olive, the origin of CFs (Fig. 1). We have also established an *in vivo* assay system in which molecules of interest are inactivated in PCs or inferior olivary neurons by lentivirus-mediated RNAi knockdown. By using these screening systems, we expect to identify candidate molecules for retrograde signaling. In addition, we will also examine whether and how the endocannabinoid 2-arachidonoylglycerol (2-AG), the best-characterized retrograde synaptic messenger in mature brain, contributes to developmental refinement of neural circuits.

【Research Methods】

In the olivo-cerebellar coculture preparation *in vitro*, we will knock down candidate molecules in PCs by lentiviral vectors with micro-RNAs designed against the molecules. We will examine CF innervations of PCs by electrophysiological methods and identify candidate molecules that may mediate retrograde signaling. Then, we will check whether the candidate molecules are involved in CF synapse elimination *in vivo*. Then, we search for molecules that are present in CFs or astroglia and interact with the retrograde signals from PCs. We will also examine how the deficiency of the identified molecules affects neural circuit activity *in vivo* by using the knockout mice of respective genes. As for the possible contribution of 2-AG in neural circuit refinement, we will examine CF synapse elimination and neural circuit maturation in the cortex in the knockout mice of 2-AG signaling molecules.

【Expected Research Achievements and Scientific Significance】

Through the comprehensive researches covering molecular, cellular and neural circuit levels, we expect to elucidate the mechanisms of retrograde signaling for the establishment of functional neural circuits during postnatal development.

【Publications Relevant to the Project】

・Uesaka N, Mikuni T, Hashimoto K, Hirai H, Sakimura K, Kano M: Organotypic coculture preparation for the study of developmental synapse elimination in mammalian brain. **J Neurosci** 32:11688-11699, 2012.
・Mikuni T, Uesaka N, Okuno H, Hirai H, Deisseroth K, Bito H, Kano M: Arc/Arg3.1 is a postsynaptic mediator of activity-dependent synapse elimination in the developing cerebellum. **Neuron** 78: 1024-1035, 2013

【Term of Project】

FY2013-2017

【Budget Allocation】

425,400 Thousand Yen

【Homepage Address and Other Contact Information】

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