DIVISION OF MOLECULAR NEUROBIOLOGY

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We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system, mainly using the visual system of the chick and mouse. This research covers many developmental events including the patterning of the retina, axonal navigation, branching and targeting, synapse formation, refinement and plasticity, and axonal regeneration. The scope of our interests also encompasses mechanisms for various functions of the mature brain, including sensation, emotion, behavior, learning and memory.

I. Mechanisms for the regional specification in the developing retina

Topographic maps are a fundamental feature of neural networks in the nervous system. Understanding the molecular mechanisms by which topographically ordered neuronal connections are established during development has long been a major challenge in developmental neurobiology. The retinotectal projection of lower vertebrates including birds has been used as a readily accessible model system. In this projection, the temporal (posterior) retina is connected to the rostral (anterior) part of the contralateral optic tectum, the nasal (anterior) retina to the caudal (posterior) tectum, and likewise the dorsal and ventral retina to the ventral and dorsal tectum, respectively. Thus, images received by the retina are precisely projected onto the tectum in a reversed manner.

Regional specification along the nasotemporal and dorsoventral axes precedes the topographic retinotectal projection in the developing retina. To understand the molecular basis of topographic retinotectal projection, an overall view of the asymmetrically expressed molecules in the developing retinas is needed. In the past ten years, we have been devoting our efforts to searching for molecules with asymmetrical distribution in the embryonic chick retina, and to characterization of their roles in the topographic retinotectal projection.

We performed a large-scale screening using differential hybridization and restriction landmark cDNA scanning (RLCS) on the embryonic day 8 (E8) chick retina. RLCS is a cDNA display system, in which a large number of cDNA species are displayed as two-dimensional spots with intensities reflecting their expression levels in mRNA. We detected ~200 spots that gave different signal intensities between the nasal and temporal retinas or between the dorsal and ventral retinas. The asymmetric expression of each gene was verified by Northern blotting and *in situ* hybridization. By subsequent analyses using molecular cloning, DNA sequencing, and database searching, 33 asymmetric molecules along the nasotemporal (N-T) axis and 20 along the dorsoventral (D-V) axis were finally identified.

Their expression profiles revealed by *in situ* hybridization are highly diverse and individual. Moreover, some of them begin to be expressed in the retina from the early developmental stages (peaking before E6), suggesting that they are implicated in the establishment (and maintenance) of regional specificity in the developing retina. We have already described some novel molecules in published papers, but the study to understand the functions of the remaining molecules and the overall hierarchical order to establish the regional specificity in the retina is currently in progress.

II. Mechanisms for the topographic retinotectal projection

In the chick embryos, the first retinal ganglion cells become postmitotic at day E2 and their axons leave the retina at E3. The earliest axons arrive at the most anterior part of the tectum at day E6 and advance over its surface in the posterior direction. These retinal axons form the stratum opticum (SO), which covers the entire tectum at E12.

At the onset, the chick retinotectal projection has a vague topographic order, and axonal sprouting begin predominantly at the vicinity and towards the site of normal terminal zone (TZ). In all vertebrates studied, however, the developing axons make trial branches, many of which are not entirely within the retinotopic area, and elimination of ectopic branches and elaboration of appropriate branches is shaped in an activity-dependent manner (Figure 1).

We have already identified a battery of molecules in the region-specific molecules in the retina (peaking after E8) that show abnormal axonal targeting, branching or synaptic arborization, when their expression was experimentally enhanced or suppressed *in vivo*. We expect that molecular mechanisms underlying the branching, pruning, and synapse formation of retinal axons shall be revealed through studies on these molecules.

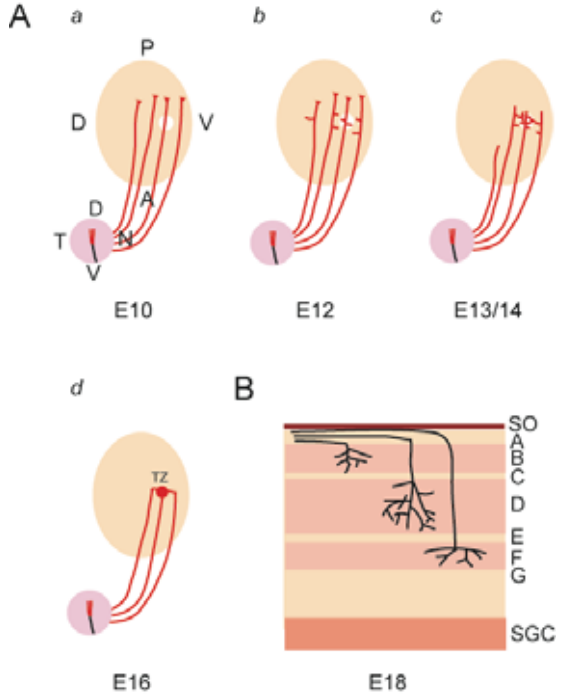

Figure 1. Development of retinotectal projection. A, Developmental processes of the topographic projection of dorsal retinal axons. TZ, terminal zone. B, Layer-specific synapse formation of retinal axons in the tectum.

III. Physiological roles of protein tyrosine phosphatase receptor type Z (Ptprz)

Protein tyrosine phosphorylation plays crucial roles in various biological aspects including brain development and brain functions. PTPζ/RPTPβ/Ptprz, a nervous system-rich receptor-type PTP, is expressed as a chondroitin sulfate proteoglycan in the brain from the early developmental stage to adulthood in neurons as well as astrocytes. This suggests that this molecule plays variegated roles in the brain development and brain function.

Ptprz binds various cell adhesion molecules (Nr-CAM, L1, contactin, NCAM and TAG-1) and extracellular matrix molecules (tenascin-C/R). In addition to these molecules, we revealed that Ptprz binds pleiotrophin (PTN)/HB-GAM and midkine (MK), closely related heparin-binding growth factors which share many biological activities. The chondroitin sulfate portion of Ptprz is essential for the high affinity binding to these growth factors.

We recently developed a genetic method named “yeast substrate-trapping system” to screen for substrates of PTPs. This method carries the advantage that not only substrates but also continuously interacting molecules are capable of being identified at the same time. By this screening, we identified Rho-family GTPase related molecules like p190 RhoGAP, Git1 (ArfGAP) and Pist as substrates, and PDZ-domain containing molecules like PSD-95 as continuously interacting proteins. The expression profile and interacting molecules suggested that Ptprz is implicated in synaptic plasticity in the hippocampus.

We then examined phenotypes of mutant mice deficient in *Ptprz* using electrophysiological, pharmacological, and behavioral approaches. Mutant mice exhibit enhanced long-term potentiation (LTP) in the CA1 region of hippocampal slices and impaired spatial learning abilities in an age-dependent manner (Figure 2): Young adult (<10 weeks old) mutant mice show normal LTP and learning abilities in the Morris water maze task, whereas adult (>13 weeks old) mutant mice exhibit enhanced LTP and impairment in the task.

The enhanced LTP is specifically canceled out by pharmacological inhibition of Rho-associated kinase (ROCK), a major downstream effector of Rho. These findings suggest that the lack of *Ptprz* leads to aberrant activation of ROCK and resultantly to enhanced LTP in the slice and learning impairments in the animal.

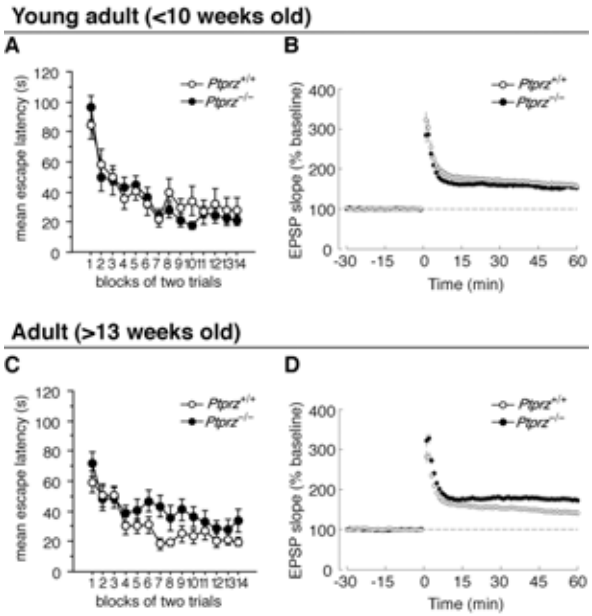

Figure 2. Age-dependent impairment of spatial learning and enhancement of hippocampal long-term potentiation (LTP) in *Ptprz*-deficient mice. A and C, Hidden-platform task of Morris water maze test. B and D, The time course of LTP in CA1 region of hippocampal slices. Tetanic stimulation (100 Hz for 1 s) was applied at 0 min. *Ptprz*-deficient mice exhibited a slower rate of spatial learning specifically at the age of >13 weeks, but not at the age of <10 weeks, which was in accordance with the age-dependent enhancement of LTP.

IV. Mechanisms for Na^+-level sensing in the brain and body fluid homeostasis

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluids in mammals. For Na homeostasis of the body, controls of Na and water intake and excretion are of prime importance. However, the system for sensing the Na level within the brain that is responsible for the control of Na- and water-intake behavior has not been fully elucidated.

We reported previously that the Na_x channel is preferentially expressed in the circumventricular organs

(CVOs) in the brain, and that *Na*$_x$ knock-out mice do not stop ingestion of saline under dehydrated conditions. Subsequently, we demonstrated that Na_x is a Na-level-sensitive Na channel. Last year, we showed that the subfornical organ (SFO), one of the CVOs, is the center of the control of salt-intake behavior in the brain, where the Na-level-sensitive Na_x channel is involved in sensing the physiological increase in the Na level of body fluids.

This year, we dissected the subcellular localization of Na_x. Double-immunostaining and immunoelectron microscopic analyses revealed that Na_x is exclusively localized to perineuronal lamellate processes extended from ependymal cells and astrocytes in the organs. In addition, glial cells isolated from the SFO were sensitive to an increase in the extracellular sodium level, as analyzed by an ion-imaging method (Figure 3). Glial cells bearing Na_x channel are thus the first to sense a physiological increase in the level of sodium in the body fluid, and regulate the neural activity of the CVOs by enveloping neurons.

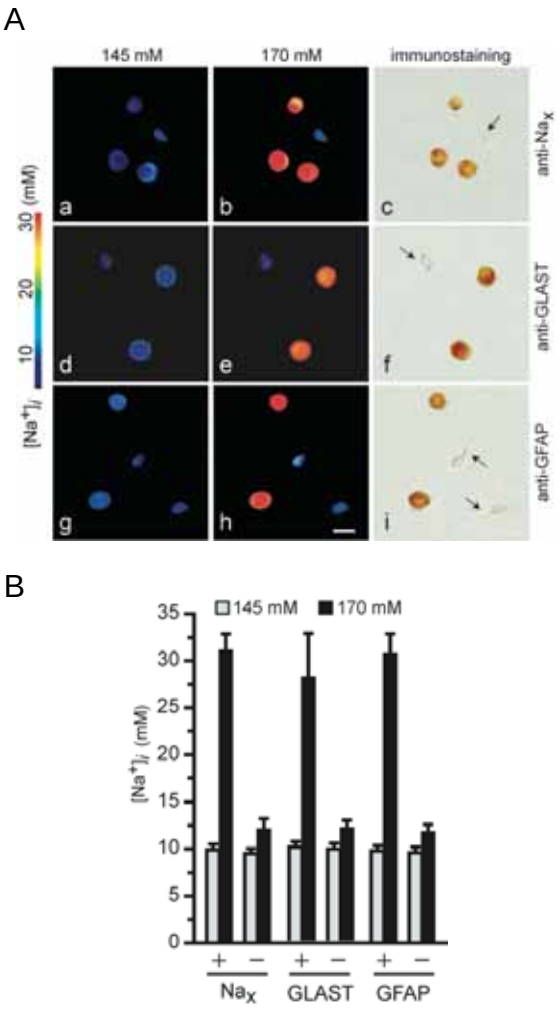

Figure 3. A, Glial cells isolated from the SFO express Na_x channel and show sensitivity to the extracellular sodium level. Pseudocolor images of the intracellular sodium concentration ($[Na^+]_i$) of SFO cells in the control solution and in the high sodium solution. A-*b*, -*e*, -*h* are images 20 min after stimulation with the hypertonic 170 mM $[Na^+]$ solution. After sodium-image recordings, cells were fixed and stained with anti-Na_x (A-*c*), anti-GLAST (A-*f*) or anti-GFAP (A-*i*) antibodies. All the sodium-sensitive cells are immunopositive for Na_x, GLAST and GFAP, indicating that these cells are glial cells. Small cells bearing short neurites (arrows) in A-*c*, -*f* and -*i* appear to be neurons, which are all insensitive to the extracellular sodium increase. Scale bar: 20 μm. B, Quantified intracellular sodium-ion concentrations before (open bars) and after (filled bars) the stimulation in Na_x-positive (+) or -negative (-) cells, in GLAST-positive (+) or -negative (-) cells, and in GFAP-positive (+) or -negative (-) cells. Data represent mean and SE (*n*=20, each).

Publication List:

Original papers

Fukada, M., Kawachi, H., Fujikawa, A., and Noda, M. (2005). Yeast substrate-trapping system for isolating substrates of protein tyrosine phosphatases: Isolation of substrates for protein tyrosine phosphatase receptor type z. Methods *35*, 54-63.

Niisato, K., Fujikawa, A., Komai, S., Shintani, T., Watanabe, E., Sakaguchi, G., Katsuura, G., Manabe, T., and Noda, M. (2005). Age-dependent enhancement of hippocampal long-term potentiation and impairment of spatial learning through the Rho-associated kinase pathway in protein tyrosine phosphatase receptor type Z-deficient mice. J. Neurosci. *25*, 1081-1088.

Noda, M., and Hiyama, T.Y. (2005). Sodium-level-sensitive sodium channel and salt-intake behavior. Chem. Senses *30* (Supple. 1), i44-i45.

Watanabe, E., Hiyama, T.Y., Shimizu, H., Kodama, R., Hayashi, N., Miyata, S., Yanagawa, Y., Obata, K., and Noda, M. (2005). Sodium-level-sensitive sodium channel Na_x is expressed in glial laminate processes in the sensory circumventricular organs. Am. J. Physiol. – Regul. Integr. Comp. Physiol. *Epub ahead of print*, PMID: 16223844.

Review articles

Fukada, M., and Noda, M. (2005). Yeast substrate-trapping system for isolating substrates of protein tyrosine phosphatases. Methods in Mol. Biol., in press.

Noda, M. (2005). The subfornical organ, a specialized sodium channel, and the sensing of sodium levels in the brain. The Neuroscientist, in press.