Department of Basic Medical Sciences

Division of Neuronal Network
神経ネットワーク分野

Professor	Toshiya Manabe, M.D., Ph.D.	教　授　医学博士	真　鍋　俊　也
Associate Professor	Yuko Sekino, Ph.D.	准教授　医学博士	関　野　祐　子
Assistant Professor	Ayako M. Watabe, Ph.D.	助　教　医学博士	渡　部　文　子
Assistant Professor	Yuji Kiyama, Ph.D.	助　教　医学博士	城　山　優　治

Our major research interest is the molecular mechanisms of higher brain functions in mammals such as emotion, and learning and memory. We are especially focusing on the roles of functional molecules localized in synapses, for instance, neurotransmitter receptors, signal transduction molecules and adhesion molecules, in neuronal information processing. We are examining receptor functions, synaptic transmission and plasticity, and their roles in the whole animal with electrophysiological, biochemical, molecular genetic and behavioral approaches.

1. Characteristic inhibitory modulation of synaptic plasticity in the dentate gyrus of the rat hippocampus

Fumiko Arima-Yoshida, Ayako M. Watabe and Toshiya Manabe

The hippocampus is essential for the formation of certain types of memory, and synaptic plasticity such as long-term potentiation (LTP) is widely accepted as a cellular and molecular basis of hippocampus-dependent memory. Although LTP in both perforant path-dentate gyrus (DG) granule cell and CA3-CA1 pyramidal cell synapses is similarly dependent on activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors, several reports suggest that modulation of LTP by γ-aminobutyric acid (GABA) receptor-mediated inhibitory inputs is stronger in perforant path-DG granule cell synapses. However, little is known about how different the mechanism and physiological relevance of the GABAergic modulation of LTP induction among different brain regions are. We confirmed that the action of $GABA_A$-receptor antagonists on LTP was more prominent in the DG, and explored the mechanism introducing such difference by examining two types of inhibition, synaptic and tonic inhibition, caused by $GABA_A$ receptors. As synaptic inhibition, we compared inhibitory versus excitatory monosynaptic responses and their summation during an LTP-inducing stimulus, and found that the balance of the summated postsynaptic currents was biased toward inhibition in the DG. As tonic inhibition, or sustained activation of extrasynaptic $GABA_A$ receptors by ambient GABA, we measured the change in holding currents of the postsynaptic cell induced by $GABA_A$-receptor antagonists, and found that the tonic inhibition was significantly stronger in the DG. Taken together, our results suggest that both the larger tonic inhibition and the larger inhibitory/excitatory summation balance during conditioning are involved in the stronger inhibition of LTP in the DG.

2. Functional contributions of synaptically localized NR2B subunits of the NMDA receptor to synaptic transmission and long-term potentiation in the adult mouse central nervous system

Hideki Miwa, Masahiro Fukaya[1], Ayako M. Watabe, Masahiko Watanabe[1] and Toshiya Manabe: [1]Department of Anatomy, Hokkaido University School of Medicine

The NMDA-type glutamate receptor is a heteromeric complex composed of the NR1 and at least one of NR2 subunits. Switching from the NR2B to NR2A subunit is thought to underlie functional alteration of the NMDA receptor during synaptic maturation, and it is generally believed that it results in preferential localization of NR2A subunits on the synaptic site and that of NR2B subunits on the extracellular site in the mature brain. It has also been proposed that activation of the NR2A and NR2B subunits results in long-term potentiation (LTP) and long-term depression (LTD), respectively. Furthermore, recent reports suggest that synaptic and extrasynaptic receptors may have distinct roles in synaptic plasticity as well as in gene expression associated with neuronal death. Here, we have investigated whether NR2B subunit-containing receptors are present and functional at mature synapses in the lateral nucleus of the amygdala (LA) and the CA1 region of the hippocampus, comparing their properties between the two brain regions. We have found, in contrast to the above hypotheses, that the NR2B subunit significantly contributes to synaptic transmission as well as LTP induction. Furthermore, its contribution is greater in the LA than in the CA1 region, and biophysical properties of NMDA receptors and the NR2B/NR2A ratio are different between the two brain regions. These results indicate that NR2B subunit-containing NMDA receptors accumulate on the synaptic site and responsible for the unique properties of synaptic function and plasticity in the amygdala.

3. Non-Hebbian synaptic plasticity physiologically induced in the hippocampus

Hiroyuki Kato, Ayako M. Watabe and Toshiya Manabe

Modern theories on memory storage have mainly focused on Hebbian long-term potentiation (LTP), which requires coincident activation of pre- and postsynaptic neurons for its induction. In addition to Hebbian LTP, the roles of non-Hebbian plasticity have also been predicted by some neuronal network models. However, still only a few pieces of evidence have been presented for the presence of such plasticity. In this study, we show in mouse hippocampal slices that LTP can be induced by postsynaptic repetitive depolarization alone in the absence of presynaptic inputs. The induction was dependent on voltage-dependent calcium channels (VDCCs) instead of N-methyl-D-aspartate receptors (NMDARs), while the expression mechanism was shared with conventional NMDAR-dependent LTP. During the potentiation, the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) was increased, suggesting novel neuron-wide nature of this form of LTP. Furthermore, we also successfully induced LTP with trains of action potentials, which supported physiological relevance of the depolarizing pulse-induced LTP. Based on these findings, we suggest a model in which neuron-wide LTP works in concert with synapse-specific Hebbian plasticity to help information processing in memory formation.

4. Behavioral analysis of Plexin-A2 knockout mice

Yuji Kiyama, Fumikazu Suto[2], Hajime Fujisawa[3] and Toshiya Manabe: [2]Department of Developmental Neurobiology, Tohoku University Graduate School of Medicine, [3]Division of Biological Science, Nagoya University Graduate School of Science

Hippocampal mossy fibers project preferentially to the stratum lucidum, the proximal-most lamina of the suprapyramidal region of the CA3 region in the hippocampus. Type A plexins can directly show repulsive activities, and all type A plexins (plexin-A1, -A2, -A3, and -A4) are expressed in the developing hippocampal system, suggesting their involvement in neuronal wiring in the hippocampus. In our previous study, we found that the projection of mossy fibers was disrupted in mutant mice for semaphorin receptors, plexin-A2 and plexin-A4. Mossy fibers were projected widely within CA3 in plexin-A4 mutant animals, while failed to invade the suprapyramidal region and instead were shifted to the infrapyramidal region and the stratum pyramidalis in plexin-A2 mutant animals. We also reported that the plexin-A2 loss-of-function phenotype was genetically suppressed by Sema6 A loss-of-function. Based on these results and results of cell biological approaches, we provided a model for the lamina-restricted projection of mossy fibers, that is, mossy fibers are endowing with plexin-A4 and thereby suppressed to invade the Sema6A-expressing suprapyramidal region of CA3, but can grow into the proximal parts of the region where Sema6A activities are masked by plexin-A2. In this study, we performed behavioral experiments and found that plexin-A2 mutant mice exhibited enhanced hippocampus-dependent spatial reference memory and spatial pattern separation tested by the

8-arm radial maze task. These results suggest that the pattern of synaptic inputs in the CA3 region determines the spatial learning ability.

5. Use-dependent amplification of presynaptic Ca^{2+} signaling by axonal ryanodine receptors at the hippocampal mossy fiber synapse

Hidemi Shimizu[1], Masahiro Fukaya[1], Miwako Yamasaki[1], Masahiko Watanabe[1], Haruyuki Kamiya[4] and Toshiya Manabe: [4]Department of Neurobiology, Hokkaido University School of Medicine

Presynaptic Ca^{2+} stores have been suggested to regulate Ca^{2+} dynamics within the nerve terminals at certain types of the synapse. However, little is known about their mode of activation, molecular identity, and detailed subcellular localization. Here, we show that the ryanodine-sensitive stores exist in axons and amplify presynaptic Ca^{2+} accumulation at the hippocampal mossy fiber synapses, which display robust presynaptic forms of plasticity. Caffeine, a potent drug inducing Ca^{2+} release from ryanodine-sensitive stores, causes elevation of presynaptic Ca^{2+} levels and enhancement of transmitter release from the mossy fiber terminals. The blockers of ryanodine receptors, TMB-8 or ryanodine, reduce presynaptic Ca^{2+} transients elicited by repetitive stimuli of mossy fibers, but do not affect those evoked by single shocks, suggesting that ryanodine receptors amplify presynaptic Ca^{2+} dynamics in an activity-dependent manner. Furthermore, we generated the specific antibody against the type 2 ryanodine receptor (RyR2; originally referred to as the cardiac type), and examined the cellular and subcellular localization using immunohistochemistry. RyR2 is highly expressed in the stratum lucidum of the CA3 region and mostly co-localizes with axonal marker NF160 but not with terminal marker VGLUT1. Immunoelectron microscopy revealed that RyR2 is distributed around smooth ER within the mossy fibers, but is almost excluded from their terminal portions. These results suggest that axonal localization of RyR2 at sites distant from the active zones enables use-dependent Ca^{2+} release from intracellular stores within the mossy fibers, and thereby facilitates robust presynaptic forms of plasticity at the mossy fiber-CA3 synapse.

6. Dual inhibition of SNARE complex formation by tomosyn ensures controlled neurotransmitter release

Toshiaki Sakisaka[5], Yasunori Yamamoto[5], Michiko Nakamura, Kouki Nishikawa[6], Hiroyoshi Ishizaki[7], Miki Okamoto-Tanaka[7], Jun Miyoshi[7], Yoshinori Fujiyoshi[6], Yoshimi Takai[8] and Toshiya Manabe: [5]Division of Membrane Dynamics, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, [6]Department of Biophysics, Kyoto University Graduate School of Science, [7]Department of Molecular Biology, Osaka Medical Center for Cancer and Cardiovascular Disease, [8]Division of Molecular and Cellular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine

Neurotransmitter release upon SNARE complex-mediated synaptic vesicle fusion is spatially and temporally regulated in presynaptic terminals. Tomosyn is known to bind syntaxin-1 through the C-terminal VAMP-like domain and thereby inhibits formation of the SNARE complex. Here, we found another inhibitory action of tomosyn against the SNARE function. The N-terminal WD-40 repeats domain of tomosyn had an intrinsic ability to catalyze oligomerization of the SNARE complex. Reduced oligomerization of the SNARE complex in tomosyn knockout mice increased probability of neurotransmitter release, suggesting that the oligomerized SNARE complex has an inhibitory effect on the neurotransmitter release. These results indicate that tomosyn inhibits the SNARE-dependent synaptic vesicle fusion by both the N-terminal WD-40 repeats domain-mediated oligomerization of the SNARE complex and the C-terminal VAMP-like domain-based competitive inhibition of the SNARE complex formation, resulting in potent inhibition of neurotransmitter release.

7. Ablation of NMDA receptors enhances the excitability of hippocampal CA3 neurons

Fumiaki Fukushima[9], Kazuhito Nakao[9], Toru Shinoe, Masahiro Fukaya[1], Shin-ichi Muramatsu[10], Kenji Sakimura[11], Hirotaka Kataoka[9], Hisashi Mori[9], Masahiko Watanabe[1], Masayoshi Mishina[9] and Toshiya Manabe: [9]Department of Molecular Neurobiology and Pharmacology, Graduate School of Medicine, University of Tokyo, [10]Division of Neurology, Department of Medicine, Jichi Medical University, [11]Department of Cellular Neurobiology, Brain Research Institute, Niigata University

Synchronized discharges in the hippocampal CA3 recurrent network are supposed to underlie network oscillations, memory formation and seizure generation. In the hippocampal CA3 network, NMDA receptors are abundant at the re-

current synapses but scarce at the mossy fiber synapses. We generated mutant mice in which NMDA receptors were abolished in hippocampal CA3 pyramidal cells by postnatal day 14. The histological and cytological organizations of the hippocampal CA3 region were indistinguishable between control and mutant mice. We found that mutant mice lacking NMDA receptors selectively in CA3 pyramidal cells became more susceptible to kainate-induced seizures. Consistently, mutant mice showed characteristic large EEG spikes associated with multiple unit activities, suggesting enhanced synchronous firing of CA3 neurons. The electrophysiological balance between fast excitatory and inhibitory synaptic transmission was comparable between control and mutant pyramidal cells in the hippocampal CA3 region, while the NMDA receptor-slow after-hyperpolarization coupling was diminished in the mutant neurons. In the adult brain, inducible ablation of NMDA receptors in the hippocampal CA3 region by the viral expression vector for Cre recombinase also induced similar large EEG spikes. Furthermore, pharmacological blockade of CA3 NMDA receptors enhanced the susceptibility to kainate-induced seizures. These results raise an intriguing possibility that hippocampal CA3 NMDA receptors may suppress the excitability of the recurrent network as a whole *in vivo* by restricting synchronous firing of CA3 neurons.

8. Requirement of the immediate early gene vesl-1S/homer-1a for fear memory formation

Naoko Inoue[12], Harumi Nakao[13], Minoru Matsui, Fumihiko Hayashi[12], Kazuki Nakao[14], Atsu Aiba[13], Kaoru Inokuchi[12] and Toshiya Manabe: [12]Mitsubishi Kagaku Institute of Life Sciences, [13]Division of Molecular Genetics, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, [14]CREST, Japan Science and Technology Agency

The formation of long-term memory and the late phase of long-term potentiation (L-LTP) depend on macromolecule synthesis, translation, and transcription in neurons. *vesl-1S* (*V*ASP/*E*na-related gene upregulated during seizure and *L*TP, also known as *homer-1a*) is an LTP-induced immediate early gene. The short form of Vesl (Vesl-1S) is an alternatively spliced isoform of the *vesl-1* gene, which also encodes the long form of the Vesl protein (Vesl-1L). Vesl-1L is a postsynaptic scaffolding protein that binds to and modulates the metabotropic glutamate receptor 1/5 (mGluR1/5), the IP3 receptor, and the ryanodine receptor. Vesl-1 null mutant mice show abnormal behavior, which includes anxiety- and depression-related behaviors, and an increase in cocaine-induced locomotion; however, the function of the short form of Vesl in behavior is poorly understood because of the lack of short-form-specific knockout mice. In this study, we generated short-form-specific gene targeting (KO) mice by knocking in part of *vesl-1L/homer-1c* cDNA. Homozygous KO mice exhibited a normal spine number and morphology. Using the contextual fear conditioning test, we demonstrated that memory acquisition and short-term memory were normal in homozygous KO mice. In contrast, these mice showed impairment in fear memory consolidation. Furthermore, the process from recent to remote memory was affected in homozygous KO mice. Interestingly, reactivation of previously consolidated fear memory attenuated the conditioning-induced freezing response in homozygous KO mice, which suggests that the short form plays a role in fear memory reconsolidation. General activity, emotional performance, and sensitivity to electrical footshock were normal in homozygous KO mice. These results indicate that the short form of the Vesl family of proteins plays a role in multiple steps of long-term, but not short-term, fear memory formation.

9. Critical involvement of tyrosine phosphorylation of the NR2A subunit of NMDA receptors in depression-related behavior

Sachiko Taniguchi[15], Takanobu Nakazawa[15], Tohru Tezuka[15], Kazumasa Yokoyama[15], Takeshi Inoue[15], Yuji Kiyama, Hiroko Izumi-Nakaseko, Ayako M. Watabe, Shigeru Kakuta[16], Katsuko Sudo[16], Yoichiro Iwakura[16], Hisashi Umemori[17], Tadashi Yamamoto and Toshiya Manabe: [15]Division of Oncology, Department of Cancer Biology and [16]Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, [17]Molecular & Behavioral Neuroscience Institute and Department of Biological Chemistry, University of Michigan Medical School

Growing evidence implicates the glutamate signaling in depression, though the molecular mechanism by which the glutamate signaling regulates depression-related behavior has remained unclear. Here, we provide evidence suggesting that tyrosine phosphorylation of the NMDA receptor, an ionotropic glutamate receptor, contributes to depression-related behavior. The NR2A subunit of the NMDA receptor is tyrosine-phosphorylated, with Tyr-1325 as its major phosphorylation site. We have generated mice expressing mutant NR2A with Tyr-1325-

Phe (Y1325F) mutation. The homozygous knockin mice show significantly less immobility in the tail suspension test and the forced swim test. In the striatum of the knockin mice, DARPP-32 (dopamine- and cAMP-regulated phosphoprotein, 32kD) phosphorylation at Thr-34, involved in regulation of depression-related behavior, is increased. We also show that Tyr-1325 phosphorylation site is required for Src-induced potentiation of the recombinant NMDA receptor channel. These data argue that Tyr-1325 phosphorylation regulates the NMDA receptor channel properties to modulate the NMDA receptor-mediated downstream signaling and depression-related behavior.

10. Kinase-dead knock-in mouse revealed an essential role of CaMKIIα kinase activity in dendritic spine enlargement, LTP and learning

Yoko Yamagata[18], Shizuka Kobayashi, Tatsuya Umeda[19], Akihiro Inoue[19], Hiroyuki Sakagami[20], Masahiro Fukaya[1], Masahiko Watanabe[1], Nobuhiko Hatanaka[21], Masako Totsuka[14], Takeshi Yagi[22], Kunihiko Obata[23], Keiji Imoto[18], Yuchio Yanagawa[24] Shigeo Okabe[25] and Toshiya Manabe: [18]Department of Information Physiology, National Institute for Physiological Sciences, [19]Department of Cell Biology, Tokyo Medical and Dental University, [20]Department of Anatomy, Kitasato University School of Medicine, [21]Division of System Neurophysiology, National Institute for Physiological Sciences, [22] Graduate School of Frontier Biosciences, Osaka University, [23]Laboratory of Neurochemistry, National Institute for Physiological Sciences, [24]Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, [25]Department of Cellular Neurobiology, Graduate School of Medicine, University of Tokyo

Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) is an essential mediator of activity-dependent synaptic plasticity that possesses multiple protein functions. So far, the autophosphorylation site-mutant mice targeted at T286 and at T305/306 have demonstrated the importance of the autonomous activity and Ca^{2+}/calmodulin-binding capacity of CaMKIIα, respectively, in the induction of long-term potentiation (LTP) and hippocampus-dependent learning. However, kinase activity of CaMKIIα, the most essential enzymatic function, has not been genetically dissected yet. Here, we generated a novel CaMKIIα knock-in mouse that completely lacks its kinase activity by introducing K42R mutation, and examined the effects on hippocampal synaptic plasticity and behavioral learning. In homozygous CaMKIIα (K42R) mice, kinase activity was reduced to the same level as in CaMKIIα null mice, while CaMKII protein expression was well preserved. Tetanic stimulation failed to induce not only LTP, but also sustained dendritic spine enlargement, a structural basis for LTP, at the Schaffer collateral-CA1 synapse, while activity-dependent postsynaptic translocation of CaMKIIα was preserved. In addition, CaMKIIα (K42R) mice showed a severe impairment in inhibitory avoidance learning, a form of memory that is dependent on the hippocampus. These results demonstrate that kinase activity of CaMKIIα is a common critical gate controlling structural, functional and behavioral expression of synaptic memory.

11. Imaging of hippocampal neuronal activity with new long-wavelength voltage-sensitive dyes

Yuko Sekino, Michelle Z.L. Kee[26,27], Joseph P. Wuskell[28], Leslie M. Loew[28] and George J. Augustine[26,27,29]: [26]Laboratory of Synaptic Circuitry, Duke-NUS Graduate Medical School, [27] A*STAR/Duke-NUS Neuroscience Research Partnership, Institute of Cell and Molecular Biology, [28]Richard D. Berlin Center for Cell Analysis and Modeling, University of Connecticut Health Center, [29]Department of Neurobiology, Duke University Medical Center

Voltage-sensitive dye (VSD) imaging of membrane potential changes is a powerful tool for the mapping of functional circuits in brain tissues. This approach overcomes one limitation of conventional electrophysiological recordings because it can monitor the activity of many neurons at once. Improvement of instrumentation, such as sensors for detecting small changes in fluorescent intensity and high-capacity processing of large amounts of imaging data, allow optical analysis of neural activity at a millisecond time scale and with sufficient spatial resolution. Thus, VSD imaging has recently become a most promising neurobiological method. Another recent and promising method is channelrhodopsin -2 (ChR2)-assisted photostimulation, which allows excitable cells to be stimulated optically. ChR2 is a light-gated, cation-selective ion channel isolated from the green algae *Chlamydomonas reinhardtii*. Because ChR2-based photostimulation can precisely excite neurons at a single-input level, much current interest is directed towards the expression of ChR2 in brain tissues to optically control neuronal activity and, thereby, to map the distribution of synaptic inputs. The combination of VSDI and ChR-assisted optical

stimulation will enable input-output mapping in neural circuits of brain tissue. VSDs typically are organic compounds that bind to cell membranes and shift their absorption and/or fluorescence emission spectra according to the transmembrane potential. Most VSDs are excited at relatively short wavelengths, which overlap with the excitation wavelengths of light-activated proteins used to control neuronal activity, such as channelrhodopsin-2. This spectral overlap is the biggest problem to be solved to permit combination of VSD imaging and photostimulation. To overcome this problem, we have assessed the utility of five new long-wavelength fluorescent VSD for imaging the activity of populations of neurons in mouse brain slices. Although all the five were capable of detecting activity resulting from activation of the Schaffer collateral-CA1 pyramidal cell synapse, they differed significantly in their properties, most notably in the signal-to-noise ratio of the changes in dye fluorescence associated with neuronal activity. Two of these dyes, Di-2-ANBDQPQ and Di-1-APEFEQPQ, should prove particularly useful for imaging activity in brain tissue and for combining VSD imaging with the control of neuronal activity via light-activated proteins such as channelrhodopsin-2 and halorhodopsin.

12. Roles of the actin cytoskeleton in dendritic spines of neurons: morphological and synaptic maturation

Hideto Takahashi[30,31], Hiroyuki Yamazaki[30], Kenji Hanamura[30], Yuko Sekino and Tomoaki Shirao[30]: [30]Department of Neurobiology and Behavior, Gunma University Graduate School of Medicine, [31]ERCGSM, Gunma University Graduate School of Medicine

Many neurological disorders are characterized by abnormalities in the structure of dendritic spines, the postsynaptic structures that receive excitatory inputs. To study the relationship between abnormal dendritic spine structure and synaptic transmission, we focused on the development of spines of cultured hippocampal pyramidal neurons. In these neurons, pharmacological blockade of the component of glutamatergic transmission mediated by AMPA receptors (AMPARs) increases the number of headless and thin protrusions in dendrites. In contrast, blockade of NMDA receptors or metabotropic glutamate receptors did not produce such changes in spine structure. Because drebrin, a spin-rich actin-binding protein, is known to govern the critical first step of dendritic spine maturation, we examined clustering of drebrin in dendritic spines. AMPAR blockade suppressed postsynaptic clustering of drebrin without affecting presynaptic clustering of synapsin I. Furthermore, depletion of drebrin with small interfering RNA treatment caused the appearance of thin, headless spines despite the presence of normal postsynaptic AMPAR activity. These data suggest that abnormal spine morphology results from suppression of AMPAR-mediated clustering of drebrin. The dynamics of drebrin clusters was explored by photobleaching individual spines. We found that AMPAR activity increased the stable fraction of drebrin, without affecting the exchange rate or the total amount of drebrin in spines. Because an increase in the stable drebrin fraction corresponds to an increase in drebrin clustering, our findings indicate that AMPAR-mediated stabilization of drebrin plays an important role in spine maturation. It is known that the loss of drebrin is found in patients with Alzheimer's disease, mild cognitive disorders and Down's syndrome. Further, experimental reduction of drebrin expression *in vivo* causes behaviors related to schizophrenia. Given this pattern of drebrin involvement in clinical disorders, our current findings suggest that altered synapse morphology in the brains of many neurological patients could be caused by changes in synapse activity that lead to changes in drebrin and in the actin cytoskeleton. Thus, therapeutic enhancement of AMPAR-mediated drebrin stabilization may be a promising strategy for treating many neurological conditions.

13. Neurogenesis in adult brain: expression of drebrin E in migrating neuroblasts in adult rat brain

Mingqiao Song[30], Nobuhiko Kojima[30], Kenji Hanamura[30], Yuko Sekino, Hiroshi K. Inoue[32], Masahiko Mikuni[33] and Tomoaki Shirao[30]: [32]Institute of Neural Organization, [33]Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine

Migrating neuroblasts in the adult brain form the rostral migratory stream (RMS) from the lateral ventricle to the olfactory bulb (OB) and then differentiate in the OB. In this study, we immunohistochemically analyzed drebrin expression in the RMS of the adult rat brain. Although drebrin is concentrated in dendritic spines of mature neurons, drebrin-immunopositive (DIP) cell bodies were observed in the RMS. The polysialated form of a neural cell adhesion molecule (PSA-NCAM) was detected in DIP cells. Ki-67, a marker of proliferating cells, was also detected in a subset of DIP cells; however, neither glial fibrillary acidic pro-

tein, nestin, nor vimentin was detected in DIP cells. These results indicate that DIP cells in the RMS are migrating neuroblasts. An image subtraction method, based on using anti-pan-drebrin and anti-drebrin A antibodies, demonstrated that DIP migrating neuroblasts are immunopositive for drebrin E but not for drebrin A (E＋A-). Furthermore, olfactory bulbectomy increased the number of cells with drebrin E＋A - signals in the RMS, indicating that these cells migrate along the RMS. Drebrin E＋A- cells were also found in the subgranular layer of the dentate gyrus and in the piriform cortex. Thus, detection of drebrin E＋A- signals is useful for identifying migrating neuroblasts in the adult brain. In the OB, drebrin E＋A- signals were observed in the cell bodies of migrating neuroblasts in the core region; however, only fibrous and punctate drebrin E＋A- signals were observed in postmigratory neuroblasts at the outer layers. These data demonstrate that the disappearance of drebrin E＋A- signals from the cell body coincides with the cessation of neuronal migration. The disappearance of drebrin E from the cell body may be a molecular switch for the cessation of migration in newly generated neuroblasts.

Publications

Miwa, H., Fukaya, M., Watabe, A.M., Watanabe, M. and Manabe, T. Functional contributions of synaptically localized NR2B subunits of the NMDA receptor to synaptic transmission and LTP induction in the adult mouse CNS. J. Physiol. (Lond.) 586: 2539-2550, 2008.

Shimizu H., Fukaya, M., Yamasaki, M., Watanabe, M., Manabe, T. and Kamiya, H. Use-dependent amplification of presynaptic Ca^{2+} signaling by axonal ryanodine receptors at the hippocampal mossy fiber synapse. Proc. Natl. Acad. Sci. USA 105: 11998-12003, 2008.

Sakisaka, T., Yamamoto, Y., Mochida, S., Nakamura, M., Nishikawa, K., Ishizaki, H., Okamoto-Tanaka, M., Miyoshi, J., Fujiyoshi, Y., Manabe, T. and Takai, Y. Dual inhibition of SNARE complex formation by tomosyn ensures controlled neurotransmitter release. J. Cell Biol. 183: 323-337, 2008.

Fukushima, F., Nakao, K., Shinoe, T., Fukaya, M., Marumatsu, S.-i., Sakimura, K., Kataoka, H., Mori, H., Watanabe, M, Manabe, M. and Mishina, M. Ablation of NMDA receptors enhances the excitability of hippocampal CA3 neurons. PLoS ONE 4: e3993, 2009.

Inoue, N., Nakao, H., Migishima, R., Hino, T., Matsui, M., Hayashi, F., Nakao, K., Manabe, T., Aiba, A. and Inokuchi, K. Requirement of the immediate early gene vesl-1S/homer-1a for fear memory formation. Mol. Brain 2: 7, 2009.

Kee, M.Z., Wuskell, J.P., Loew, L.M., Augustine, G.J. and Sekino, Y.(C.A.) Imaging activity of neuronal populations with new long-wavelength voltage-sensitive dyes. Brain Cell Biol. 36: 157-172, 2008.

Takahashi, T., Yamazaki, H., Hanamura, K., Sekino, Y. and Shirao, T. AMPA receptor inhibition causes abnormal dendritic spines by destabilizing drebrin. J. Cell Sci. 122: 1211-1219, 2009.

Song, M., Kojima, N., Hanamura. K., Sekino, Y., Inoue, K.H., Mikuni, M. and Shirao, T. Expression of drebrin E in migrating neuroblasts in adult rat brain: coincidence between drebrin E disappearance from cell body and cessation of migration. Neuroscience 152: 670-682, 2008.

真鍋俊也．海馬におけるシナプス可塑性の分子細胞機構．蛋白質・核酸・酵素．三品昌美，山森哲雄，狩野方伸，村上富士夫，貝淵弘三編集．共立出版．53(3)：555-559，2008.

真鍋俊也．長期増強．生体の科学．医学書院．59(5)：430-431，2008.

真鍋俊也．記憶の分子メカニズム．Brain and Nerve．医学書院．60(7)：707-715，2008.

真鍋俊也．海馬におけるシナプス可塑性．分子・細胞・シナプスからみる脳．古市貞一編集．東京大学出版会．216-230，2008.

渡部文子，真鍋俊也．扁桃体シナプス長期増強と情動記憶の分子制御．Clinical Neuroscience．中外医学社．26(4)：402-405，2008.

真鍋俊也．海馬歯状回およびCA3領域における外部情報のパターン分離．Medical Briefs in Brain & Nerve．マッキャンヘルスケアーワールドワイドジャパン．16(4)：12-13，2008.