Department of Basic Medical Sciences

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

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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. Non-Hebbian synaptic plasticity induced by repetitive postsynaptic action potentials

Hiroyuki K. 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 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 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 the possible existence of depolarizing pulse-induced LTP in vivo. 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.

2. Plexin-A2 regulates spatial memory and pattern separation through structural modification of mossy fiber projection in the CA3 region of the mouse hippocampus

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The hippocampus has been implicated in certain types of memory, including spatial memory. It has been known that the distribution of

mossy fibers, axons of dentate gyrus (DG) granule cells, is modified dynamically by spatial learning in living animals. However, the precise mechanism of the regulation of mossy fiber distribution during memory formation is not well understood. We have previously reported that plexin-A2 (PLA2), one of the type A plexins that show repulsive activities to the class 6 semaphorins, regulates the distribution of mossy fiber terminals in the CA3 region and that the mutant mice lacking PLA2 ($PLA2^{-/-}$ mice) exhibit a shift of mossy fibers from the suprapyramidal to the infra- and intrapyramidal regions. In order to test whether the difference in the distribution of mossy fiber terminals affects abilities of learning and memory, we have performed extensive behavioral analyses of $PLA2^{-/-}$ mice. We found that sensorimotor functions and emotional behaviors of $PLA2^{-/-}$ mice were normal, although motor learning was markedly impaired presumably through aberrant distribution of cerebellar granule cells, and that contextual and auditory fear conditioning, which is at least partially dependent on the hippocampus, was also intact. In contrast, $PLA2^{-/-}$ mice exhibited enhanced hippocampus-dependent spatial reference memory and spatial pattern separation, which is the ability to discriminate fine differences in external environments, tested by the 8-arm radial maze task. These results suggest that the projection of mossy fibers regulated by PLA2 may be a specific determinant of the ability of spatial reference memory and pattern separation.

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

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Synchronized discharges in the hippocampal CA3 recurrent network are supposed to underlie network oscillations, memory formation and seizure generation. In the hippocampal CA3 network, NMDARs are abundant at the recurrent synapses but scarce at the mossy fiber synapses. We generated mutant mice in which NMDARs 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 NMDARs 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 NMDAR-slow after-hyperpolarization coupling was diminished in the mutant neurons. In the adult brain, inducible ablation of NMDARs in the hippocampal CA3 region by the viral expression vector for Cre recombinase also induced similar large EEG spikes. Furthermore, pharmacological blockade of CA3 NMDARs enhanced the susceptibility to kainate-induced seizures. These results raise an intriguing possibility that hippocampal CA3 NMDARs may suppress the excitability of the recurrent network as a whole *in vivo* by restricting synchronous firing of CA3 neurons.

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

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The formation of long-term memory and the late phase of 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 un-

derstood 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.

5. Involvement of tyrosine phosphorylation of the NMDAR GluN2A subunit in depression-related behavior

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Major depressive and bipolar disorders are serious illnesses that affect millions of people. Growing evidence implicates glutamate signaling in depression, though the molecular mechanism by which glutamate signaling regulates depression-related behavior remains unknown. In this study, we provide evidence suggesting that the tyrosine phosphorylation of the NMDAR, an ionotropic glutamate receptor, contributes to depression-related behavior. The GluN2A subunit of the NMDAR is tyrosine-phosphorylated, with Tyr-1325 as its one of the major phosphorylation site. We have generated mice expressing mutant GluN2A with a Tyr-1325-Phe mutation to prevent phosphorylation of this site in vivo. The homozygous knock-in mice show antidepression-like behavior in the tail suspension test and in the forced swim test. In the striatum of the knock-in mice, DARPP-32 phosphorylation at Thr-34, which is important for the regulation of depression-related behaviors, is increased. We also show that the Tyr-1325 phosphorylation site is required for Src-mediated potentiation of the recombinant NMDAR channel. These data argue that the Tyr-1325 phosphorylation regulates NMDAR channel properties and the NMDAR-mediated downstream signaling to modulate depression-related behavior.

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

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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 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.

7. Roles of the actin cytoskeleton in dendritic spine morphogenesis of hippocampal neurons

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Spine morphogenesis mainly occurs during development as a morphological shift from filopodia-like thin protrusions to bulbous ones. We have previously reported that synaptic clustering of the actin-binding protein drebrin in dendritic filopodia governs spine morphogenesis and synaptic PSD-95 clustering. Here, we report the activity-dependent cellular mechanisms for spine morphogenesis, in which the activity of AMPA receptors (AMPARs) regulates drebrin clustering in spines by promoting drebrin stabilization. In cultured developing hippocampal neurons, pharmacological blockade of AMPARs, but not of other glutamate receptors, suppressed postsynaptic drebrin clustering without affecting presynaptic clustering of synapsin I (synapsin-1). Conversely, the enhancement of the action of AMPARs promoted drebrin clustering in spines. When we explored drebrin dynamics by photobleaching individual spines, we found that AMPAR activity increased the fraction of stable drebrin without affecting the time constant of drebrin turnover. An increase in the fraction of stable drebrin corresponded with increased drebrin clustering. AMPAR blockade also suppressed normal morphological maturation of spines and synaptic PSD-95 clustering in spines. Together, these data suggest that AMPAR-mediated stabilization of drebrin in spines is an activity-dependent cellular mechanism for spine morphogenesis.

8. Fos-positive neurons in the supramammillary nucleus of the rat exposed to novel environment

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The supramammillary nucleus (SuM) in the hypothalamus is proposed to regulate the function of the hippocampus through distinct fiber connection. Several investigations suggest that the SuM is relevant to anxiety and defensive behavior. Function of the SuM, however, is not known exactly. In order to demonstrate the spatial activation of the SuM in physiologically behaving rats, we investigated Fos induction in the SuM by exposure to novel environment. To correct uneven background in microscopic preparations, we applied a convolution filter, resulting in reliable automatic counting of Fos-positive neurons and analyzed the distribution of Fos-positive neurons in the whole region of SuM. A large number of Fos-positive neurons were observed throughout the entire SuM after rats exposed to a novel open field. A three-dimensional density map revealed that density of the Fos-positive neurons was highest in the medial SuM, especially in its core regions. Based on these results we suggest that the medial SuM modulates defensive behavior and that the lateral SuM modulates emotional and memory functions of the hippocampus.

Publications

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