論文題目: Subcortical roles of NMDA receptor and adenylyl cyclase 1 in the refinement of whisker-barrel circuits in the mouse

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Elucidating the mechanisms of activity-dependent neural circuit formation is an important theme in neuroscience. It has been thought that refinements of neural circuits in mammals require neural activities. One of suitable systems to investigate mechanisms of activity-dependent neural circuit formation is the mouse whisker-barrel circuit (somatosensory system), in which tactile inputs from whiskers are conveyed to the cortex through the brainstem and thalamus. In the mouse somatosensory system, whisker-related patterns are recapitulated in the cortex, thalamus and brainstem as barrels, barreloids and barrelettes, respectively. In the cortex, thalamocortical axons (TCAs) make clusters in the center of barrels, and layer 4 neurons accumulate around TCA patches to make cylindrical patterns. These patterns are consolidated during the first postnatal week in activity-dependent manners. Previous studies of genetically modified mice have contributed to the understanding of the molecular bases of activity-dependent development of neural circuit in the neocortex. Genes that contribute to the formation of whisker-related patterns have been extensively studied in the cortex but not in the subcortex. Various molecules have been suggested to play important roles in barrel formation. Among many molecules, N-methyl-D-aspartate-type glutamate receptors (NMDA receptors) and adenylyl cyclase I (AC1) have received much attention. To reveal subcortical mechanisms in activity-dependent neural circuit formation, I have focused on these two molecules.

NMDA receptor, which is an ionotropic glutamate receptor, is a major source of calcium ion and contributes to the learning and memory. Previous studies indicate that NMDA receptor is an essential molecule for the whisker-related pattern formation. NR1 is the essential subunit of NMDA receptors. Global NR1 knockdown study indicates that all whisker-specific patterns are severely impaired in these mice. On the other hand, although cortex-specific NR1 knockout (Cx-NR1KO) mice impair barrels in the cortex, barreloids in the thalamus and barrelettes in the brainstem are normal. These results

suggest that subcortical NMDA receptors may also have roles in the whisker-related pattern formation. To elucidate the possibility that subcortical NMDA receptors contribute to the pattern formation, I first generated thalamus-specific NR1 knockout (Th-NR1KO) mice by using 5HTT-Cre mice, which express Cre recombinase specifically in the thalamus. Th-NR1KO mice showed complete impairment of barreloids in the thalamus. TCAs formed rudimentary patterns, while layer 4 neurons failed to form cylindrical patterns in the cortex. These results indicated that thalamic NMDA receptors are essential for the barreloid formation in the thalamus. Impairments of the thalamic patterns might continuously change TCA patterns in the cortex. Layer 4 neurons failed to accumulate around TCA patches in the Th-NR1KO cortex. In Th-NR1KO mice, barrelettes in the brainstem showed normal patterns, although previous global NR1 knockdown study indicates complete loss of barrelettes.

Next, to examine the role of brainstem NMDA receptors, I generated brainstem-specific NR1 knockout (Bs-NR1KO) mice by using Krox-20 Cre mice (a kind gift of Dr. Patrick Charnay), which express Cre recombinase specifically in the rhombomere 3 and 5. Bs-NR1KO mice had no barrelettes in the brainstem. On the other hand, partial impairment of barreloids and barrels were observed in the thalamus and the cortex, respectively, of these mice. These results indicated that brainstem NMDA receptors have critical roles for the formation of barrelettes. Furthermore, these results suggested that NMDA receptors contribute to the pattern formation in the region where they are expressed. Pattern impairments in the region lacking NMDA receptors continuously affected the patterning of projecting axons. For example, Th-NR1KO mice lost barreloids, and projecting axons from the thalamus (TCAs) failed to form precise whisker-related patterns in the cortex. Thus, post-synaptic NMDA receptors were essential for the refinement of whisker-related pattern.

A previous study revealed that when NMDA receptor function is reduced globally, all the patterns of whisker-barrel circuits are lost. Furthermore, another study showed that when all excitatory cortical neurons lack functional NMDA receptors, the cortical pattern is lost. These and other studies suggest that NMDA receptor signaling is important in patterning of whisker-barrel circuit throughout the somatosensory system. Here I answered the question whether selective deletion of either thalamus or

brainstem NMDA receptors results in impairment of cortical patterns. In Th-NR1KO mice, barrelettes were normal but barreloids were absent. It was predicted that the consequent absence of cortical barrels should be observed in Th-NR1KO mice. Unexpectedly, barrels that correspond to large whiskers formed rudimental patterns in the cortex although barreloids in the thalamus were completely lost. How TCAs formed whisker-related patterns in the cortex even when the pattern in thalamus was disrupted remains unsolved. As described previously, it is possible that NMDA receptor-independent mechanisms also participate in patterning of TCA terminals. Examples of these mechanisms include axonal sorting by various guidance molecules such as Ephrin A5, Netrin1, Sema3A/3F. These guidance molecules determine targeting region of projecting axons in thalamocortical pathway in NMDA receptor-independent manners.

Cortex-specific NR1KO and single cell NR1KO studies showed that NMDA receptors are essential for dendritic orientation of layer 4 neurons. A recent study of BTB/POZ domain containing 3 (BTBD3) indicates that BTBD3 translocates from soma to cell nuclei in activity-dependent manners and regulates dendritic orientation of cortical cells. It is thought that this neuronal activity might be derived from NMDA receptor-dependent $Ca^{2+}$-influx into neurons. BTBD3 makes dendrite of layer 4 cells toward the active TCA cluster during developmental stage. Furthermore, ectopic expression of BTBD3 also makes cell dendrite to extend toward the active axonal terminals in the other cortical region in some biological species. These results suggest that BTBD3 might lie on the downstream of NMDA receptor-mediated neural activity in the cortex during development.

It was surprising that Bs-NR1KO mice had some barrels in the cortex, although they had impaired brainstem barrelettes. It is thought that barrels and barreloids are copies of whisker-related patterns in the brainstem barrelettes. If the upstream patterns of cortical barrels in the brainstem barrelettes and the thalamus barreloids are absent, the deficiencies of cortical patterns should be caused. But I found that Bs-NR1KO mice had some barrels in the cortex. It is possible that the topographical order remained in the brainstem even though there was no whisker-related pattern formation. Another possibility is that novel mechanisms act in sorting of projecting axons in the somatosensory system.

AC1 is also implicated in activity-dependent refinement of neural circuits. AC1 is triggered by Ca2+/CaM signaling to synthesize cyclic AMP (cAMP) from ATP. cAMP is well known as a second messenger in cell signaling. Global AC1 knockout (AC1KO) mice show a lack of spatial segregation of TCAs into individual barrels. This result indicates a critical role of AC1 for the barrel formation. However, because AC1 is expressed throughout the somatosensory system, the sites where AC1 operates for circuit refinement remain to be identified. In a previous study, cortex-specific AC1 knockout (Cx-AC1KO) mice showed grossly normal barrels and barreloid in the cortex and thalamus, respectively. These results suggest that subcortical AC1 may have important roles for the pattern formation in the whisker-barrel circuit. To examine the roles of subcortical AC1, I generated thalamus-specific AC1 knockout (Th-AC1KO) mice, using 5-HTT Cre mice. Barrels in Th-AC1KO mice were severely impaired, and the border of individual barrels was difficult to be distinguished in the cortex. On the other hand, barreloids in the thalamus showed normal patterns in Th-AC1KO mice. These results revealed that thalamic AC1 have important roles for barrel formation but not for barreloid formation. These results suggested that presynaptic AC1 has important roles in the axonal patterning in their target region.

AC1 and cAMP signaling have been central in studies of molecular mechanisms of neuronal circuit refinement in early postnatal development since "*barrelless*" mutant was found. "*Barrelless*" mutant mice show complete loss of cortical barrels and partial impairment of thalamic barreloids. Spontaneous mutant and global AC1KO mice clearly indicate that AC1 is an essential molecule for the barrel formation. It remained unclear whether AC1 functions in the presynaptic TCA or postsynaptic cortical neurons in the barrel patterning. In a previous study of Cx-AC1KO mice, cortical patterns were grossly normal. Furthermore, cortex and partial thalamus AC1KO (Cx/pTh-AC1KO) results showed burred and unclear patterns in the cortex corresponding to the anterior snout. These results indicate that cortical AC1 is dispensable for the barrel formation in the developmental stage. It is thought that the other type of Ca2+-stimulated adenylyl cyclase, adenylyl cyclase 8 (AC8), may contribute to the barrel formation. But cortical AC1 and AC8 KO studies also indicate that the $Ca^{2+}$/CaM-stimulated adenylyl cyclases in the cortex are not important for the barrel formation. Here I showed that both Th-AC1KO and Bs-AC1KO mice failed to make discrete patterns of

TCAs and trigemino-thalamic axons (TTAs). These results indicated that presynaptic AC1 in the whisker-barrel circuit is essential for the clustering of projecting axons in the target region. In mammalian visual system, recent evidence by in vitro and in vivo studies suggest that presynaptic retinal AC1 is important for the postnatal refinement of retinotectal and retiongeniculate projections. Our results in the somatosensory whisker-barrel circuit are consistent with their results in the visual system. In somatosensory system, as in visual system, presynaptic AC1 is important for the whisker-related pattern formation.

These studies reveal roles of subcortical genes in the activity-dependent pattern formation in mouse somatosensory system.

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