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(別紙様式 2)
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論文内容の要旨
Summary of thesis contents

Elucidating the mechanisms of activity-dependent neural circuit formation is an important issue in neuroscience. It is thought that refinement of neural circuits in mammals requires neural activity. One of a suitable system to investigate the mechanism of activity-dependent neural circuit formation is the mouse whisker-barrel circuit (somatosensory system). Tactile inputs from whiskers are conveyed to the cortex through the brainstem and the 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 and make cylindrical patterns. These patterns are consolidated during the first postnatal week in activity-dependent manners. Cortical genes that contribute to the formation of whisker-related patterns have been extensively studied, but subcortical genes are not well understood. I am interested in revealing subcortical mechanisms in activity-dependent neural circuit formation. Here I tested 2 molecules, N-methyl-D-aspartate-type glutamate receptors (NMDA receptors) and adenylyl cyclase 1 (AC1), both of which have been implicated in activity-dependent refinement of neural circuits.

NMDA receptor is an inotropic glutamate receptor, which mediates excitatory synaptic transmission in the brain. NMDA receptors are a major calcium ion source and contribute to the learning and memory. NR1 is the essential subunit of NMDA receptors. Previous studies indicate that NMDA receptor is an essential molecule for the whisker-related pattern formation. Global NMDA receptor knockdown study indicates that all whisker-specific patterns are severely impaired. On the other hand, although cortex-specific

NR1 knockout (Cx-NR1KO) mice impaired barrels in the cortex, barreloids in the thalamus and barrelettes in the brainstem were 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 thalamus-specific Cre recombinase expression mice, 5HTT-Cre mice. Th-NR1KO mice showed complete impairment of barreloids in the thalamus. TCAs form rudimentary patterns, while layer 4 neurons completely fail to form cylindrical patterns in the cortex. These results indicate that thalamic NMDA receptors are essential for the barreloid formation in the thalamus. Defection 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 receptor, I generated brainstem-specific NR1KO (Bs-NR1KO) mice by using brainstem-specific Cre recombinase expression mice, Krox-20 Cre mice. Bs-NR1KO mice appeared pattern loss in the brainstem. On the other hand, partial defection of barreloids and barrels were observed in the thalamus and the cortex, respectively. These results indicate that brainstem NMDA receptors have critical roles for the formation of barrelettes. Furthermore, these results suggest that NMDA receptors contribute to the pattern formation in the region where they are expressed. Pattern impairment in the region lacking NMDA receptors continuously affects the patterning of projecting axons. For example, Th-NR1KO mice lost their barreloids, and projecting axons from thalamus (TCAs) failed to form precise whisker-related patterns

in the cortex.

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 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 role of subcortical AC1, I generated thalamus-specific AC1KO (Th-AC1KO) 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 reveal that thalamic AC1 have important roles for barrel formation but not for barreloid formation. To explain phenotypic discrepancy between global AC1KO mice and Th-AC1KO mice, I generated brainstem-specific AC1KO (Bs-AC1KO) mice. Bs-AC1KO mice displayed abnormality of barreloid formation in the thalamus. These results clearly indicate that presynaptic AC1 has important roles in the axonal patterning in their target region.

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

(別紙様式 3)
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博士論文の審査結果の要旨

Summary of the results of the doctoral thesis screening

マウス終脳の体性感覚皮質にはBarrelとよばれる特徴的な組織学的構造が存在する。Barrelの個々のモジュールは、口吻の洞毛１本１本に対応しており、感覚刺激が誘起する神経活動に依存して形成される。洞毛からの感覚入力は、脳幹と視床の２つの中継地点でシナプスを乗り換えて終脳皮質に到達するが、各々の中継地点にもBarrelette(脳幹)、Barreloid(視床)とよばれるBarrel様のモジュール構造が存在する。鈴木亜友美さんは、脳幹→視床→皮質という神経回路の流れにそってBarrel様の構造が構築されるしくみについて、神経活動を制御する２つの遺伝子、NMDA型グルタミン酸受容体サブユニットNR1とtype-1 adenylyl cyclase (AC1)の条件的遺伝子破壊マウスを用いて研究した。

これまでの研究で、NR1遺伝子を全身で破壊した場合には、脳幹、視床、皮質のすべてのBarrel様構造が消滅し、皮質特異的に破壊した場合には、皮質のポストシナプス細胞のBarrel配置だけが異常になることが示されていた。鈴木さんは、NR1を視床特異的に破壊すると、視床のBarreloid構造が消失し、それに伴い皮質のBarrel構造形成も激しく障害されることを見いだした。さらに鈴木さんは、NR1を脳幹特異的に破壊し、脳幹のBarrelette構造が完全に消失する事を明らかにした。このマウスでは、視床のBarreloidも皮質のBarrelも形成不全を示すが、上位のレベルにいくほど回復傾向が認められ、Barrel様構造の形成原理を考える上で大変興味深い。

AC1遺伝子のほうは、これまでの研究で、全身で破壊した場合には皮質のBarrelが完全欠損するが、皮質特異的に破壊した場合には、ほぼ正常にBarrelが形成されることが示されており、AC1は視床軸索ではたらくと予想されていた。鈴木さんは、実際にAC1遺伝子を視床特異的に破壊して、その視床での機能が皮質のBarrel形成に必須であることを示した。このマウスの場合、上記のNR1遺伝子とは異なり、視床のBarreloid自体は正常に形成されるが、ここから伸長するする軸索が皮質内でBarrel状に分離しない。したがって、AC1は視床軸索で前シナプス的に機能すると考えられた。鈴木さんはAC1の脳幹特異的破壊も行い、脳幹から視床へ伸びる軸索のBarreloid状の分離が異常になる事を明らかにした。したがってここでも、AC1の前シナプス的機能が示唆された。

以上の研究は、Barrel形成の理解においてこれまで決定的に欠けていた穴を埋めるものであり、回路形成の分子機構について重要な示唆を与えるものである。研究成果はすでに、２報の原著論文として発表および受理されており、博士号授与の要件を満たすと審査員全員一致で判断した。