主論文の要旨

Mice with exonic *RELN* deletion identified from a patient with schizophrenia have impaired visual discrimination learning and reversal learning in touchscreen operant tasks

（統合失調症患者から同定された *RELN* エキソン遺伝子欠失を有するマウスはタッチスクリーン型オペラント課題における視覚弁別学習および逆転学習に障害を有する）

名古屋大学大学院医学系研究科　総合医学専攻
臨床医薬学講座　医療薬学分野
（指導：山田 清文　教授）
廖 婧竹

【Introduction】

The Reelin gene (*RELN*) encodes a large extracellular protein, which has multiple roles in brain development and adult brain function. Reelin activates a series of neuronal signal transduction pathways in the adult brain that function in synaptic plasticity, dendritic morphology, and cognitive function. To further investigate the roles of *Reln* in brain function, we generated a mouse line using the C57BL/6J strain with the specific *Reln* deletion identified from a Japanese patient with schizophrenia (*Reln*-del mice). These mice exhibited abnormal sociality, but the pathophysiological significance of the *Reln* deletion for higher brain functions, such as learning and behavioral flexibility remains unclear. In this study, cognitive function in *Reln*-del mice was assessed using touchscreen-based visual discrimination (VD) and reversal learning (RL) tasks.

【Object】

Reln-del mice were generated by the CRISPR/Cas9 method in a C57BL/6J genetic background. Heterozygous *Reln*-del mice were generated by intercrossing *Reln*-del males and C57BL/6J females. Littermate wild-type C57BL/6J mice (WT mice) were used as controls, 7- to 8-week-old WT (male n = 6, female n = 5) and *Reln*-del (male n = 6, female n = 6) mice were used in experiments.

【Method】

The touchscreen-based VD and RL tasks were performed using the touchscreen chamber system. In the VD task, a pair of visual stimuli was presented simultaneously at a pseudorandom location on the screen. Touching the correct stimulus resulted in a liquid reward (20 μl of milk), whereas touching the incorrect response resulted in a 5-second time-out punishment and started with a correction trial. The RL task was similar to the VD task described above, except that the reward contingencies were reversed. The criterion of the VD and RL task was mice reaching a correct response rate of greater than 80% in two consecutive sessions.

【Results】

In the pretraining and simple VD task, there was no difference in the number of total sessions to reach the criterion between WT and *Reln*-del mice, suggesting that visual discrimination learning of *Reln*-del mice in the simple VD task was not impaired. The results of simple RL task revealed that in the early perseverative phase, no significant differences were observed between two genotypes. In the later learning phase, however, *Reln*-del mice showed impaired performance, suggesting that behavioral flexibility was unaffected but later learning was impaired by the deletion of *Reln* in the simple RL task.

Next, in the complex VD task, *Reln*-del mice were significantly slower to reach the

learning criterion by daily training than WT mice, suggesting that visual discrimination learning was significantly impaired in *Reln*-del mice when the visual stimuli became more complicated. In the complex RL task, *Reln*-del mice were significantly slower to reach the criterion than WT mice. Analysis of early perseverative and later learning phases revealed that in the early perseverative phase, no significant difference was observed between two genotypes. However, in later learning phase, *Reln*-del mice showed impaired performance. These results suggested that *Reln*-del mice showed continuation of VD impairment in the complex RL task.

【Discussion】

In the present study, we evaluated the ability of learning and behavioral flexibility in *Reln*-del mice using touchscreen-based behavioral tasks. *Reln*-del mice exhibited no impaired performance in pretraining, suggesting that their visuospatial and motor functions were normal. No significant differences were observed in the performance of the simple VD and RL task, but *Reln* deletion impaired the performance in complex VD task and RL task. When reversal sessions were divided according to whether performance was < 50% correct (early perseverative phase) or ≥ 50% correct (later learning phase), a significant impairment was evident in the later learning phase but not early perseverative phase in *Reln*-del mice. Thus, *Reln*-del mice may have some impairments of visual discrimination learning in the complex VD task, simple RL task and complex RL task.

Previous reports showed that normal performance in VD task depends on the intact function of the corticostriatal circuitry, which is essential for learning behaviors in humans, nonhuman primates, and rodents. Previous report showed that c-Fos expression increased in dorsal striatum (DS) during choice learning and relearning, suggested DS was increasingly activated during these two stages. Thus, DS is critical for choice learning. Furthermore, in the reversal session, the c-Fos expression increased specifically in the orbitofrontal cortex (OFC) and ventromedial prefrontal cortex (vmPFC), which means the OFC and vmPFC were active during choice shifting. RL task is employed as a measure of perseveration which has been found to be dependent upon OFC function. No significant difference in early perseverative phase suggests that OFC may be unaffected by the deletion of *Reln*. On the other hand, *Reln*-del mice showed impairment in learning and relearning phases. Thus, it is possible that the cognitive dysfunction in *Reln*-del mice may be associated with the dysfunction in the DS.

【Summary】

In conclusion, this is the first report that associative learning and behavioral flexibility are impaired in *Reln*-del mice. Our study suggests that Reelin plays an important role in cognition, and *Reln*-del mice will enable us to examine the neurobiological mechanisms

underlying the cognitive dysfunction caused by the deletion of *Reln* and therapeutic strategies.