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学 位 論 文 題 目	遺伝子改変マウスの行動解析と小脳における最初期遺伝子マッピングによる運動課題遂行に関与する脳機能の解析
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論文内容の要旨

Summary of thesis contents

Motor control and motor learning have been studied for many years, but there are still many unsolved questions. To understand the brain mechanisms underlying motor functions, a behavioral experimental system, the “step-wheel system” has been developed in my laboratory. In this system, a mouse must adjust their limb control to the pegs (footholds) in the motor-driven wheel to drink water. The peg arrangement can be changed by the experimenters. A previous study in my laboratory suggested that neuronal nitric oxide synthase (nNOS)-positive interneurons in the striatum show specific changes in neuronal activity depending on the peg pattern. This finding indicates that these interneurons have certain roles in motor learning. I examined the effects of blocking the NOS function on motor performance in the step-wheel system using nNOS knockout (KO) mice and NOS inhibitors. The nNOS KO and wild-type (WT) mice showed no difference in *Touch Time* (index of missed steps), but nNOS KO mice showed higher scores in *Water On Time* (index of drinking) and *Turn Stops* (number of times the wheel stopped turning, whereby mice could not keep running). I considered that the results of the nNOS KO mice were not due to improved motor functions but activated motivation. In support of this, the injection of NOS inhibitor to the striatum did not affect these parameters. These results led me to study the motor function of other types of genetically altered mice using the step-wheel system. I then studied the roles of the striatal direct- and indirect-pathways in the motor function using dopamine D1 receptor (D1R) KO and D2 receptor (D2R) KO mice. The striatal direct pathway and the indirect pathway facilitates and inhibits movements, respectively, during motor control. These two projection neurons express two different dopamine receptor subtypes, that is, striatonigral (direct pathway) neurons express D1R and striatopallidal (indirect pathway) neurons express D2R. I compared the performance of these KO mice using the step-wheel and the rota-rod task (a commonly used apparatus for the motor task in rodents). Both KO mice showed a poorer motor function than the WT mice. However, it was difficult to evaluate the exact motor ability of D2R KO mice in rota-rod tasks because they showed high performance from the beginning and did not show improvement. However, using the step-wheel system, I can clearly distinguish the motor leaning ability among three genotypes of mice in the order of D1R KO < D2R KO < WT mice. It is reported that D1R KO mice are deficient in reward-driven tasks, and D2R KO mice are deficient in aversive tasks. In regard to this report, my results suggest that, by using the rota-rod and step-wheel tasks together, we will be able to evaluate a greater variety of motor abilities in genetically engineered mice. To understand the possible interaction between the striatum and cerebellum, in the third series of experiments, I examined

whether there are any differences in cerebellar neuronal responses during motor tasks in D1R KO, D2R KO and WT mice. The immediate-early gene (IEG) can be used as a marker of neuronal activity because the visualization of IEG expression enables us to detect neuronal activities at the cellular level. A previous study in our laboratories showed that the stimuli that mimic cerebellar long-term depression (LTD) induced the expressions of *c-fos* and *jun-B* in cerebellar Purkinje cells *in vitro* and *in vivo*. I examined the cerebellar expressions of eight IEGs, namely, *c-fos*, *fos-B*, *jun-B*, *c-jun*, *jun-D*, *zif-268*, *krox 20*, and *arc*. The expressions of all eight IEGs were minimal in naive mice. In contrast, *c-fos* and *jun-B* expressions were induced in the mice that performed rota-rod tasks. The expression of *c-fos* was detected at almost all lobules (except lobule 7) in the vermis and at Crus1, Cop, and most significantly at the flocculus in the hemisphere. The expression of *jun-B* was also detected at almost the same regions as those showing *c-fos* expression but the signals were weaker and it was detected in few cells, Purkinje cells and some sparse cells in the molecular layer. These findings indicate the usefulness of *c-fos* and *jun-B* as neuronal activity markers in the cerebellum. Next, I checked the expression patterns of *c-fos* and *jun-B* in the flocculus of WT, D1R KO and D2R KO mice. Although I did not detect significant differences among these mouse genotypes, I unexpectedly observed *c-fos* and *jun-B* expressions even 24 h after the mice rode on stationary rota-rod. There may be some differences 24 h after stationary rota-rod tasks between the naive mice and mice experienced series of motor tasks. These results and meaning need more research in the future.