LABORATORY OF DIRECTOR GENERAL

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One of the largest themes of the 21st century is to promote brain research in an attempt to understand the ìmi nd.î There are many approaches to elucidating the mechanism of ìm indî function, including research into human intelligence, memory, cognition, emotion and volition. Research on developing an excellent artificial system for information processing, research on the ontogeny and the development of the brain, and research on protecting the brain from aging, neurological and psychiatric disorders are also important themes. The promotion of brain research is considered an important issue in many countries including Japan.

Our approach to understanding the brain is to explore the molecular mechanism of higher brain function regulating animal behavior by employing genetic modification of living organisms. Major research interests of the laboratory are to elucidate a physiological role of dopamine receptors in animal behavior, an implication of N-methyl-D-aspartate receptors (NMDARs) in psychiatric disorders and the roles of the *ras* family in the brain by producing genetically altered mice, both gene targeted and transgenic mice.

I. Dopamine regulates locomotion and eating behavior

The dopaminergic system is considered to be involved in locomotor control, emotional behavior, reward, motivation and thought process. Hypoactivity or hyperactivity of the dopaminergic system can result in neurological and psychiatric disorders such as Parkinsonís disease and schizophrenia. In mammals, five subtypes of dopamine receptors (D1R-D5R) are identified and classified into two major groups, D1-like (D1R, D5R) and D2-like (D2R, D3R, D4R) receptors on the basis of the gene structure and the pharmacological and intracellular signaling properties. The contribution of D1-like and D2-like receptors to behaviors is determined pharmacologically.

We generated knockout (KO) mice lacking each of five dopamine receptors and multiple KO mice lacking more than one dopamine receptor simultaneously. We focused on D1R and D2R, major subtypes of D1-like and D2-like receptors, respectively, which are most widely and abundantly expressed. We found the *D1R/D2R* double knockout (DKO) mice showed severe impairment in locomotion and feeding that was not observed in *D1R* or *D2R* KO mice. Although the *D1R/D2R* DKO mice were born normally and showed suckling behavior, the *D1R/D2R* DKO mice exhibited rapid decrease in locomotion and no initiation of eating and eventually died by the third postnatal week. These findings suggest that dopaminergic transmission via D1R or D2R is involved in neural development of the areas that are implicated in the regulation of locomotion and eating.

To examine the involvement of the dopaminergic system in the regulation of locomotion and eating we generated mutant mice in which dopaminergic transmission can be shut off at a time point of interest. We utilized a tetracycline controllable expression system and generated transgenic mice harboring conditional *D1R* expression on the *D1R/D2R* DKO background (Figure 1).

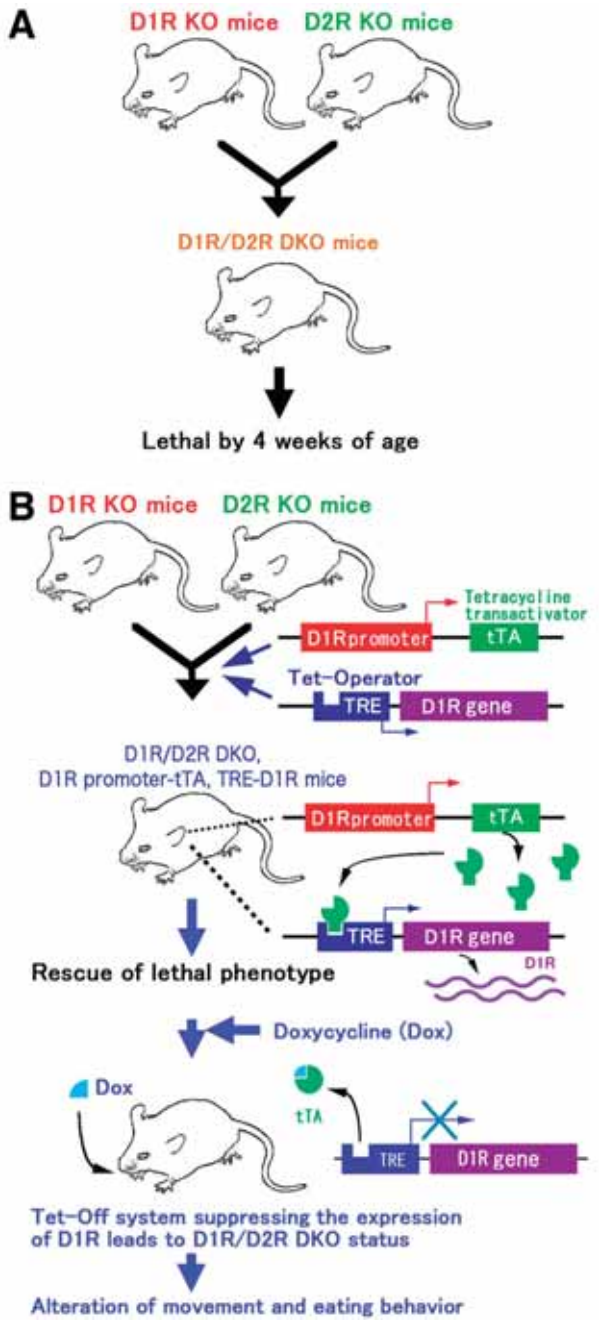

Figure 1. *D1R/D2R* DKO mice were rescued by conditional *D1R* expression. (A) *D1R/D2R* DKO mice showed impairment in locomotion and feeding and died prematurely. (B) To rescue *D1R/D2R* DKO mice, transgenic mice harboring conditional *D1R* expression on the *D1R/D2R* DKO background were generated by tetracycline controllable expression system.

We obtained several transgenic mouse lines rescuing lethal phenotype of the *D1R/D2R* DKO mice (*D1R/D2R* DKO-*D1R* rescued mice). The *D1R/D2R* DKO-*D1R* rescued mice exhibited decrease in expression level of transgene in the striatum (Figure 2) and decrease in locomotion and food/water intake by doxycycline (Dox) administration (Figure 3). These results indicate that areas harboring Dox-controllable *D1R* expression are responsible for the regulation of locomotion and eating behavior.

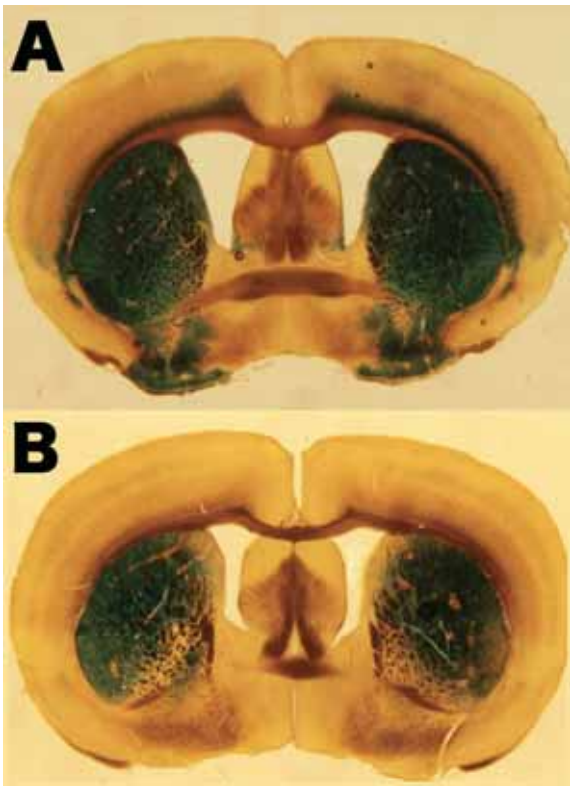

Figure 2. (A) Before doxycycline (Dox) administration the intensive expression of transgene was seen in the striatum of the *D1R/D2R* DKO-*D1R* rescued mice. (B) The amount of transgene expression was suppressed in the striatum by Dox administration. Frontal sections of mouse brains with X-gal staining were shown.

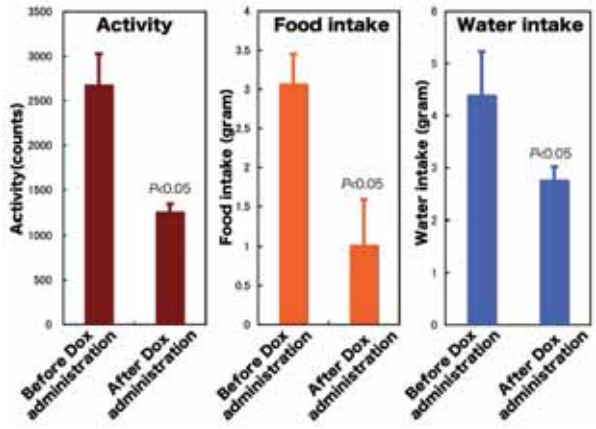

Figure 3. Alteration of locomotion, eating and drinking of *D1R/D2R* DKO-*D1R* rescued mice by Dox administration. Locomotive activity, food intake and water intake of the mice before and after Dox administration are shown.

II. Analysis of the function of NMDARs

The NMDARs are widely expressed in the nervous system, are fundamental to excitatory neurotransmission, and play a number of important roles. There are many reports on the involvement of the NMDARs in learning and memory. According to one hypothesis schizophrenia may involve a defect in NMDAR function. NMDARs consist of NR1 subunit and at least one subunit of NR2A-NR2D. We generated KO mice lacking each of NMDAR subunits and multiple KO mice lacking two subunits simultaneously, and found the *NR2A* homozygous, *NR2B* heterozygous mutant ($NR2A^{-/-}$,$NR2B^{+/-}$) mice exhibited behavioral alteration similar to that observed in patients with schizophrenia. We are developing an experimental devise in order to assess behavioral alteration of the $NR2A^{-/-}$,$NR2B^{+/-}$ mice and study the molecular mechanism relationship between the mutation of NMDAR genes and altered behavior.

III. Analysis of roles *ras* family in the brain

The *ras* proto-oncogene plays a critical role in cell growth control as a central component of mitogenic signal transduction pathways. In mammals there are H-, N-, K-*ras* identified as the *ras* family. H-, N-, K-*ras* have an overlapped spatial expression pattern as well as an overlapped function. We generated H-, N-, K-*ras* KO mice, and discovered that H-*ras* was involved in synaptic transmission and plasticity in the hippocampus and that K-*ras* was essential for normal development and involved in the survival of embryonic motoneurons. To investigate the distinct function of the individual Ras protein in the brain we generated *ras* DKO mice expressing a single Ras and triple KO mice lacking all H-, N-, and K-Ras and analyzed developmental aspects of these mutant mice.

Publication list:

Original papers

Isono, K., Nemoto, K., Li, Y., Takada, Y., Suzuki, R., Katsuki, M., Nakagawara, A., and Koseki, H. (2006). Overlapping roles for homeodomain-interacting protein kinases hipk1 and hipk2 in the mediation of cell growth in response to morphogenetic and genotoxic signals. Mol. Cell. Biol. *26*, 2758-2771.

Komine, Y., Nakamura, K., Katsuki, M., and Yamamori, T. (2006). Novel transcription factor zfh-5 is negatively regulated by its own antisense RNA in mouse brain. Mol. Cell. Neurosci. *31*, 273-283.

Muto, S., Katsuki, M., and Horie, S. (2006). Rapid induction of skin tumors in human but not mouse c-Ha-ras proto-oncogene transgenic mice by chemical carcinogenesis. Cancer Sci. *97*, 842-847.

Niimi, N., Sugo, N., Aratani, Y., Gondo, Y., Katsuki, M., and Koyama, H. (2006). Decreased mutant frequency in embryonic brain of DNA polymerase beta null mice. Mutagenesis *21*, 55-59.