論文の内容の要旨

論文題目　Enhanced locomotor activity, reduced anxiety-like behaviors and enhanced aggression of striatum-selective TrkB-deficient mouse

和訳　線条体選択的なTrkB欠損マウスにおける自発運動活性の亢進、不安様行動の減弱と攻撃性の亢進

佐藤暢彦

TrkB is the Trk family of receptor tyrosine kinases, and a high-affinity receptor for the neurotrophic factor brain-derived neurotrophic factor (BDNF). Neurotrophin signaling through this receptor regulates cell survival, proliferation, the fate of neural precursors, axon and dendrite growth and patterning, and the expression and activity of functionally important proteins, such as ion channels and neurotransmitter receptors. (Bibel *et al*., 2000; Huang *et al*., 2003) In the adult nervous system, the BDNF/TrkB signaling regulates synaptic strength, plasticity (Bibel *et al*., 2000; Huang *et al*., 2003), and plays an important role in learning and memory (Yamada *et al*., 2002), and drug addiction (Hall *et al*., 2003).

Recently, several studies have implicated BDNF/TrkB signaling in mood disorders such as anxiety and depression (Martinowich *et al*., 2007). Previous studies report that when placed in stressful settings, $BDNF^{+/-}$ and $BDNF^{Met/Met}$ mice, which impaired BDNF/TrkB signaling in the whole brain, exhibited increased anxiety-like behaviors (Chen *et al*., 2006). But whether this tendency is persistent in several brain regions including the striatum, which is an important component of the reward system associated with drug addiction, is unclear.

In the striatum, TrkB proteins are not only on the medium spiny neurons (MSNs) (Altar *et al*.,

1998), which are the predominant projection neurons in the striatum, but also on the terminal of dopaminergic neurons projecting to MSNs (Mufson *et al*., 1994). On the other hand, all of the BDNF in the striatum is present in the terminals of afferent neurons (Altar *et al*., 1997). The hippocampal, cortical, nigral, amygdala and thalamic neuron groups that project to the striatum contain high levels of BDNF mRNA (Hofer *et al*., 1990; Seroogy *et al*., 1993). Thus, both pharmacological technique and conventional knockout should have affected both pre- and postsynaptic BDNF/TrkB signalings equally.

I therefore established striatum-selective TrkB-deficient (mutant) mouse line. The G-protein γ7 subunit mRNA is expressed predominantly in medium spiny neurons of the caudate-putamen (CP) and nucleus accumbens (NAc) and neurons of the olfactory tubercle (Watson *et al*., 1994). To develop a striatal neuron-selective gene manipulation system, I chose to use the $Gng7^{Cre}$ mouse line by inserting the gene encoding Cre recombinase into the translational site of G-protein γ7 subunit gene (*Gng7*) through homologous recombination in embryonic stem cells derived from the C57BL/6 strain (Mishina and Sakimura, 2007). I crossed the $Gng7^{Cre}$ mice with the CAG-CAT-Z11 reporter mouse (Tsujita *et al*., 1999). Brain slices prepared from $Gng7^{Cre} \times$ CAG-CAT-Z11 mice were stained for β-galactosidase activity to monitor the Cre recombinase activity. Strong β-galactosidase staining was found predominantly in the CP, NAc and olfactory tubercle.

To obtain $Gng7^{Cre/+}$; $TrkB^{flox/flox}$ (mutant) and $TrkB^{flox/flox}$ (control) mice I then crossed the $Gng7^{Cre}$ (Gγ7-cre) mouse with $TrkB^{flox}$ mouse line through homologous recombination in embryonic stem cells derived from the C57BL/6 strain (Iwano, 2002).

To investigate whether Cre recombinase-dependent excision affects the expression of TrkB protein, I performed immunoblot analysis on striatal homogenates from control and mutant mice. Immunoblot analysis with anti-pan-TrkB antibody showed that a band at 145 kDa, which corresponds in molecular mass of the full-length TrkB, was decreased to about 50% in mutant mice compared with control mice. It was noticed that, because TrkB are expressed in both striatal medium spiny neurons and neurons projecting to the striatum (Merlio *et al*., 1992; Altar *et al*., 1994), TrkB of afferent terminals may be also detected. *In situ* hybridization analysis using a probe corresponding to the floxed kinase domain in collaboration with Dr. Uchigashima showed that the expression of the TrkB mRNA is diminished selectively in the striatum. Furthermore, quantitative RT-PCR (QRT-PCR) analysis showed an extensive decrease of the TrkB mRNA in the striatum of mutant mice. These results suggest that the expression of the TrkB was almost completely diminished in the striatum of the mutant mice.

During bleeding of the mutant mice, I noted unusual fighting between male control mice and mutant littermates that sometimes resulted in severe injuries. To quantify the frequency of fighting, I performed isolation-induced aggression. In comparison with littermate control mice, mutant mice manifested extensive aggression. These results suggest that the striatal TrkB is involved in the

suppression of aggressive behavior.

Since aggression frequently correlates with anxiety (Parmigiani *et al*., 1998), I suspected that the alteration of anxiety-like behaviors of mutant mice might have contributed to their enhanced aggressive behavior. To examine whether the loss of striatal TrkB was associated with anxiety-like behaviors, I performed the elevated plus-maze. The elevated plus-maze is a standard test to analyze anxiety-like behavior by measuring the avoidance of the unsheltered open arms. In comparison with littermate control mice, mutant mice exhibited a significant increase in the percentage of entries into open arms and the percentage of time spent in open arms. Mutant mice also displayed increased exploratory behavior as demonstrated by a enhanced total arm entries. I also performed the open field test, another standard measure of anxiety-like behavior that place subjects in conflict situations, but there were not significant difference between control and mutant mice in the percentage of time spent in the center square. I further investigated mutant mice in another animal model related to anxiety that is fundamentally different from the conflict model. For this purpose, I chose the marble-burying behavior test (Njung'e and Handley SL, 1991). In this test, as mice that are more anxious must engage in active behaviors (defensive marble burying) as opposed to passive behaviors utilized to avoid anxiogenic stimuli in the elevated plus-maze (Jacobson *et al*., 2007). In comparison with littermate control mice, mutant mice exhibited a significant decrease in the number of buried marbles. Mutant mice also displayed increased horizontal activity.

Taken together, these findings suggest that the BDNF/TrkB signaling in the striatum is required for the expression of the anxiety-like behaviors.

Given that reduced anxiety-like behaviors and enhanced aggressive behavior in mutant mice can be attributed to either a cognitive dysfunction in which mice could not understand the risk or a lack of proper inhibition of impulsive behaviors, I then performed the cliff-avoidance test (Yoshida *et al*., 1998; Matsuoka *et al*., 2005). In this test, control mice placed on an elevated transparent platform (the base of an inverted glass beaker with a height more than twice the mice's body length) avoided the edge and rarely fell. In contrast, nine of nine mutant mice fell from the platform within 10 min, where as three of nine control mice fell within this period. This behavioral abnormality might reflect either cognitive dysfunction or impulsivity.

Mutant mice showed a consistently increase in locomotor activity in several different tests, i.e. elevated plus-maze, open field test and marble-burying behavior test. To confirm these observations, we measured horizontal activity in the home cage for 3 days. Total distance traveled by mutant mice was significantly greater than that of control mice during the dark cycle. These results suggest that TrkB of the striatum is required for the suppressive regulation of the locomotor activity in the novel environment and during the dark cycles.

Medium spiny neurons, which account for over 90% of neurons in the striatum, are divided into two types, which give rise to the two main components of the prototypical basal ganglia circuit, the

“direct” and “indirect” striatal projection systems. Most of those neurons projecting to the lateral globus pallidus (LGP) alone (the indirect pathway) contain the neuropeptide enkephalin, whereas most of those which project to the substance nigra (SN) (the direct pathway) contain the neuropeptide sibstance P (Gerfen, 2004). These two neuropeptides are anterogradely transported to the axon terminals in the afferent regions (Drago *et* al., 1998). As one measure of the functional state of direct and indirect pathway, I performed immunohistochemical staining of a series of coronal section with anti-enkephalin and substance P antibodies. Although immunoreactivities for substance P were comparable between genotypes, immunoreactivities for enkephalin in the LGP of mutant mice were impaired. Consistently, in collaboration with Dr. Uchigashima, the expressions of the enkephalin mRNA were reduced in the striatum of the mutant mice, while those of the substance P mRNA remained intact in the mutant striatum. Furthermore, QRT-PCR analysis showed a decrease in enkephalin mRNA in the striatum of mutant mice but not substance P mRNA. Previous reports showed that mutant mice lacking enkephalin exhibited enhanced aggressive behavior (König *et al*., 1996) and lacking adenosine A_{2A} receptor showed the reduction of the enkephalin mRNA expression and enhanced aggressive behavior (Ledent *et al*., 1997). Thus, I propose that the ablation of TrkB in the medium spiny neurons of the striatum reduces the expression of enkephalin in the indirect pathway and thus results in the enhancement of the aggressive behavior.

Thus, these results suggest that the BDNF/TrkB signaling in the striatum is required for the suppressive regulation of the locomotor activity and the expression of the anxiety-like behaviors. Furthermore, the striatal TrkB is involved in the suppression of aggressive behavior probably by regulating enkephalinergic projections.