論文の内容の要旨

論文題目　A Novel Form of Memory for Auditory Fear Conditioning at a Low-Intensity Unconditioned Stimulus
（音の恐怖条件付け学習における新規記憶経路の解明）

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Fear is one of the most potent emotional experiences of our lifetime and is an adaptive component of response to potentially threatening stimuli, serving a function that is critical to the survival of higher vertebrates (Davis, 1992; LeDoux, 2000). Too much or inappropriate fear, however, accounts for many common psychiatric problems (Kent et al., 2003; Millan, 2003; Uys et al., 2003). A fearful experience can establish an emotional memory that results in permanent behavioral changes and emotional memories have been observed in many animal groups (Blanchard et al., 1993). The brain mechanisms underlying fear are similar in different species and the fear system will respond similarly in a person or a rodent, using a limited set of defense response strategies (LeDoux, 1996). The memory of learned fear can be assessed quantitatively using a Pavlovian fear-conditioning paradigm (Davis, 1992; LeDoux, 2000). During fear conditioning, an initially neutral conditioned stimulus (CS, e.g. an auditory tone) acquires biological significance by becoming associated with an aversive unconditioned

stimulus (US, e.g. a footshock). After learning this association, an animal responds to the previously neutral CS with a set of defensive behavioral responses, such as freezing. Cumulative evidence suggests that the amygdala plays a central role in the acquisition, storage and expression of fear memory.

Here, an inducible striatal neuron ablation system in transgenic mice was developed. 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-specific gene manipulation system, Gγ7-Cre and Gγ7-mCrePR mouse lines were produced by inserting the gene encoding Cre recombinase or Cre recombinase-progesterone receptor fusing protein (CrePR) into the translational initiation site of the G-protein γ7 subunit gene (*Gng7*) through homologous recombination in embryonic stem cells derived from the C57BL/6 strain (Mishina and Sakimura, 2007). Then, the Gγ7-Cre and Gγ7-mCrePR mice were crossed with the CAG-CAT-Z11 reporter mouse (Tsujita et al., 1999). Brain slices prepared from Gγ7-Cre × 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. The Gγ7-mCrePR mouse was crossed with a knock-in mouse (Eno2-STOP-DTA) in which the Cre-inducible diphtheria toxin A gene (*DTA*) was introduced into the neuron-specific enolase gene (*Eno2*) locus (Kobayakawa et al., 2007). In $Gng7^{+/mCrePR}$ mice, one allele retains the intact *Gng7* gene, and the other is inactivated by insertion of the *CrePR* gene. RU-486 was injected into the peritoneum of Gγ7-mCrePR × Eno2-STOP-DTA mice at postnatal day 42 (P42) to induce the recombinase activity of CrePR (Tsujita et al., 1999; Takeuchi et al., 2005; Mishina and Sakimura, 2007). Mock-injected mice served as controls. Thirteen days after RU-486 treatment, the ablation of striatal neurons was then

quantitatively examined by immunohistochemical staining for NeuN, a marker protein for neurons. The density of NeuN-positive neurons in the CP drastically decreased by 13 day after RU-486 injection ($F_{6,54} = 99.5$, $P < 0.001$, one-way ANOVA) and remained at a very low level thereafter. The number of NeuN-positive cells in the NAc core and shell also decreased with a similar time course. However, NeuN immunostaining signals in other brain regions including the amygdala were comparable between mock- and RU-486-treated mice. Along with the NeuN-immunohistochemistry, present results suggest that induction of CrePR-mediated DTA expression by RU-486 injection successfully ablated almost completely the medium spiny neurons that comprise approximately 90% of the NeuN-positive striatal neurons within 13 days. In subsequent analyses, I used Gγ7-mCrePR × Eno2-STOP-DTA mice from 13 to 22 days after RU-486 administration as striatal neuron-ablated mutant mice and corresponding mock-injected littermates served as controls.

Despite that approximately 90% of striatal neurons were ablated, the motor performance of the mutant mice appeared to be comparable to that of control mice in stationary thin rod ($F_{1,15} = 1.38$, $P = 0.26$, repeated measures ANOVA) and rotating rod tests ($F_{1,14} = 3.57$, $P = 0.08$, one-way ANOVA). Mutant mice were subjected to auditory fear conditioning to examine the possible involvement of striatal neurons in the formation of the emotional memory. Fourteen days after RU-486 treatment, mutant mice were trained for auditory fear conditioning. The ablation of striatal neurons hardly affected the auditory fear learning under the standard condition (control, 31.6 ± 5.1%; mutant, 28.0 ± 5.1%; $F_{1,15} = 0.27$, $P = 0.61$) in agreement with previous studies. When conditioned with a low-intensity unconditioned stimulus, however, the formation of long-term fear memory but not short-tem memory was impaired in striatal neuron-ablated mice ($P < 0.05$, mutant vs. control; $P < 0.01$, mutant vs. RU-486

control). Consistently, the ablation of striatal neurons 24 h after conditioning with the low-intensity unconditioned stimulus, when the long-term fear memory was formed, diminished the retention of the long-term memory (mock-injected mice, 37.6 ± 3.9%; RU-486-injected mice, 11.5 ± 2.6%; $F_{1,13} = 41.9$, $P < 0.001$). These results reveal a novel form of the auditory fear memory depending on striatal neurons at the low-intensity unconditioned stimulus.

In addition, I report that post-conditioning infusions of NMDA receptor inhibitors into the striatum of wild-type animals disrupt consolidation of auditory fear memory ($F_{1,18} = 5.3$, $P = 0.034$, repeated measures ANOVA) when mice were trained with a low-intensity unconditioned stimulus. Furthermore, intra-striatum infusions of protein synthesis blocker immediately ($F_{1,13} = 9.3$, $P = 0.009$, repeated measures ANOVA) or 1 hour ($F_{1,15} = 5.15$, $P = 0.038$) after the conditioning prevent auditory fear memory whereas infusions 3 hours after conditioning do not affect the freezing level ($F_{1,12} = 0.22$, $P = 0.65$), indicating that there is a critical time window of protein synthesis for memory consolidation. These findings demonstrate that NMDA receptors and *de novo* protein synthesis in the striatum are crucial for the consolidation of auditory fear memory with low-intensity shock.