博士論文（要約）

Role of dopamine in reward-oriented behavior：water drinking behavior in mice with reduced dopamine secretion

（報酬志向行動におけるドーパミンの役割：ドーパミン産生減少マウスを用いた飲水行動研究）

高　孔駿

論文の内容の要旨

論文題目 **Role of dopamine in reward-oriented behavior： water drinking behavior in mice with reduced dopamine secretion**

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Introduction:

Most animal behaviors are driven by rewards. Behaviors that are reward-oriented are also modulated in response to homeostatic regulation (Mangel & Clark, 1986; McNamara & Houston, 1986). Thus, animals constantly alter their reward-oriented behaviors when value of a cue or reward changes or when internal state (hunger and thirst) shifts (Bindra, 1978; Toates, 1986). Elucidating this process of behavioral modulation is fundamental to understanding how a decision is made. Considerable evidence has indicated involvement of mesolimbic dopamine (DA) system in reward-oriented behaviors (Flagel & Robinson, 2017; Berridge, 2018). Pharmacology studies showed that dopaminergic (DAergic) stimulation of the nucleus accumbens (NAc) triggers an intense response to obtain a reward (Fig. 1), even if a rat has undergone extinction training (Peciña & Berridge, 2013). To reveal how animal behavior is modulated, licking behavior of rodents is widely used as a means to explore this modulation from three different perspectives, liking (hedonic impact of reward), wanting (incentive motivation), and learning (reward prediction) (D'Aquila & Galistu, 2017; Dastugue *et al.*, 2018; Johnson, 2018). It has been suggested that DA receptors modulate rodent drinking behavior, but D1-like and D2-like receptors may differentially influence the incentive motivation of mice or the hedonic impact of reward (Genn *et al.*, 2003; Galistu & D'Aquila, 2013; Robles & Johnson, 2017).

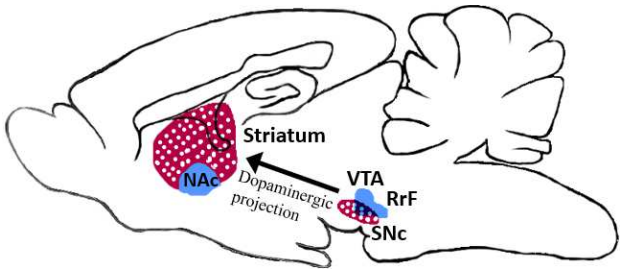

Fig. 1 Distribution of DAergic neurons and their projections in the adult mouse brain. VTA, ventral tegmental area; RrF, retrorubral field; SNc, substantia nigra pars compacta.

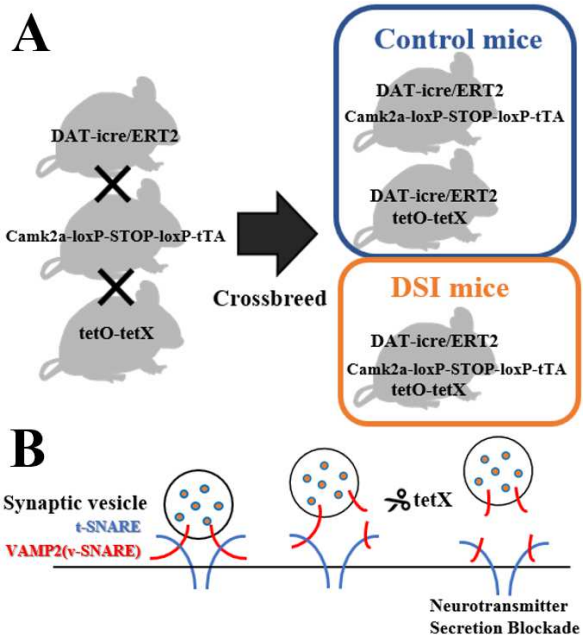

Fig. 2 (A) Breeding scheme to generate DSI mice. **(B)** The tetX prevents synaptic vesicles from releasing neurotransmitter (dopamine).

In the present study, I assessed water drinking behavior by analyzing licking microstructure (number of licks and bursts, size of bursts, and intra-burst lick speed) in a new triple transgenic mouse line that our laboratory generated. The new mouse line is expected to exhibit partial blockade of synaptic release rather than severely impaired DA secretion, and thus it was named the DA secretion interference (DSI) mouse line. To study the potential differential effects on drinking behavior and decision-making, DSI mice and control mice were treated with a DA D1 receptor agonist (A68930 or SKF38393) or a DA D2/D3 receptor agonist (ropinirole) before licking test.

Chapter 1：Behavioral Change Induced by Reduced Dopamine Secretion

DSI mice (Fig. 2A) were generated by crossbreeding tetX transgenic mouse lines (Camk2a-loxP-STOP-loxP-tetracycline transactivator [tTA] and tetO-tetanus toxin [tetX]) (Nakashiba et al., 2008) with a mouse line carrying the Slc6a3(DAT)-icre/ERT2 transgene (Schriever et al., 2017) that encodes a tamoxifen-inducible Cre recombinase under the control of a Slc6a3 promoter, which is active specifically in DAergic neurons. Tamoxifen administration removed loxP-STOP-loxP cassette in DAergic neurons by Cre-loxP recombination and resulted in the expression of tetX light chain that blocks synaptic release of DA (Fig. 2B). To confirm the cell type specificity of Cre expression by immunohistochemistry, a Cre reporter transgene (Gt(ROSA)26-Sor$^{tm1(EYFP)Cos}$) was introduced into some DSI mice. The EYFP signal triggered by the Cre-loxP recombination overlapped the marker signal (TH) of DAergic neurons (Fig. 3), confirming the cell specificity of transgene expression. Revealed by microdialysis, the DA concentration in mice administered tamoxifen was reduced to 61.4% of that in controls in the striatum and 54.5% in the NAc (Fig. 4A). DSI mice had similar body weights and water consumption as littermate controls. The spatial learning and reversal learning also remained intact in DSI mice. As the motor control impairment of DSI mice was revealed only under a challenging situation (rotating speed ≥ 28 rpm) by rotarod test (Fig. 4B) (Sidak test, Ctrl vs. DSI; day 7, p = 0.004; day 8, p = 0.021), it was concluded that the DSI mice were sufficiently fit to perform the licking test.

The numbers of total licks (Fig. 5), which indicates the degree of feeding activity, is commonly used to evaluate changes in fluid ingestion (Davis, 1989; Mendez et al., 2016). The influence of moderate DA loss on drinking behavior was investigated under conditions of water deprivation, which provides the motivation to drink. Both the control and DSI mice learned to lick the water nozzle for a water reward by the end of 7 consecutive days of training, but the moderate loss of DA resulted in fewer licks in water-deprived DSI mice (Fig. 6). As the number of licks is representative of general drinking behavior, the fewer licks by DSI mice suggests changes to the water drinking behavior.

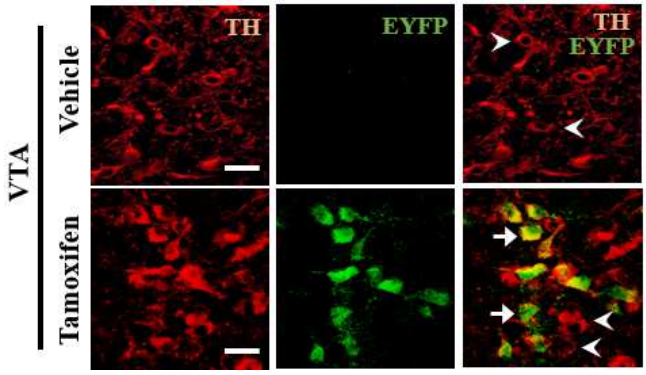

Fig. 3 TH (red) and EYFP (green) double staining in the VTA of a DSI mouse carrying a Cre reporter transgene. Scale bars, 25 μm.

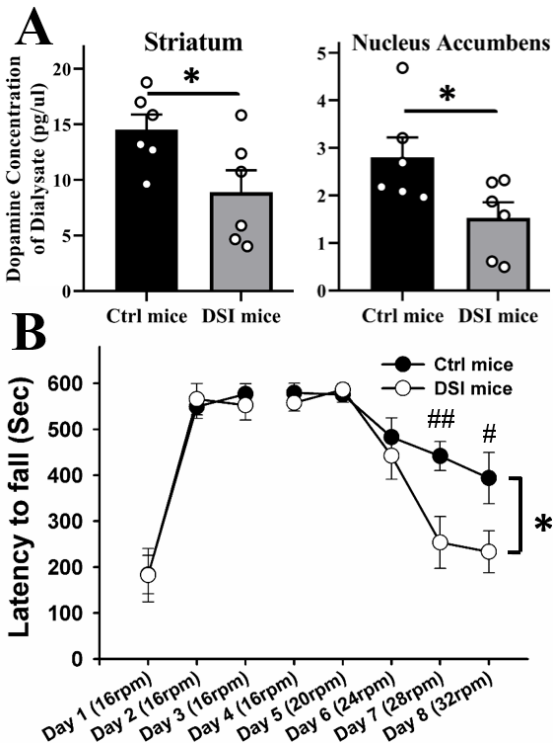

Fig. 4 (A) DA concentrations of the dialysates collected from Striatum or NAc. **(B)** Rotarod test. RM-ANOVA: *p < 0.05 compared to Ctrl mice. #p < 0.05 and ##p < 0.01 compared within a day.

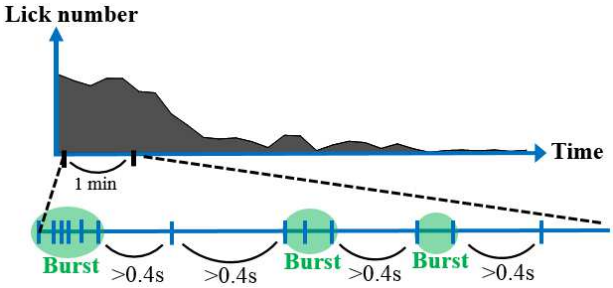

Fig. 5 Scheme of the licking microstructure.

Chapter 2：Dopamine Receptor Agonist Affects Water Drinking Behavior of Mouse Under Thirsty Condition

Rodents usually cluster their licks into separate sets known as bursts or bouts (Fig. 5) (D'Aquila & Galistu, 2017; Johnson, 2018). The number of bursts [continuous licking (≥ 2 licks) with less than 0.4 s between two licks] reflects the incentive motivation (wanting) triggered by cues because it indicates the activation of responses. The size of the bursts (number of licks within a burst) reflects the hedonic impact of reward (liking), and the intra-burst lick speed is an indicator of licking-associated motor control.

The DA D1 agonist (A68930 or SKF38393 treatment) ameliorated the drinking behavior of DSI mice by restoring the decreased lick number (Fig. 7) while the SKF38393 treatment also increased the numbers of licks in control mice. The effects of A68930 and SKF38393 may differ because of their different drug distributions in the brain and binding selectivities, and it would suggest that the effects of D1 agonists may depend on how neural circuitries are stimulated.

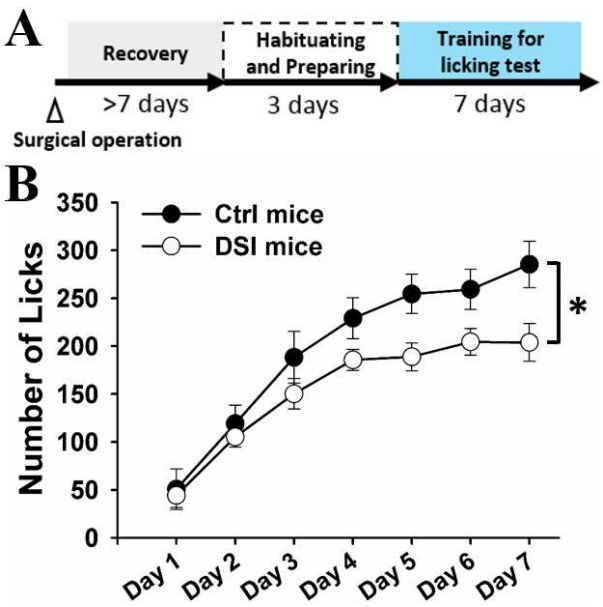

Fig. 6 (A) Scheme of the training for licking test. **(B)** Number of licks during training. RM-ANOVA: *p < 0.05 compared to Ctrl mice.

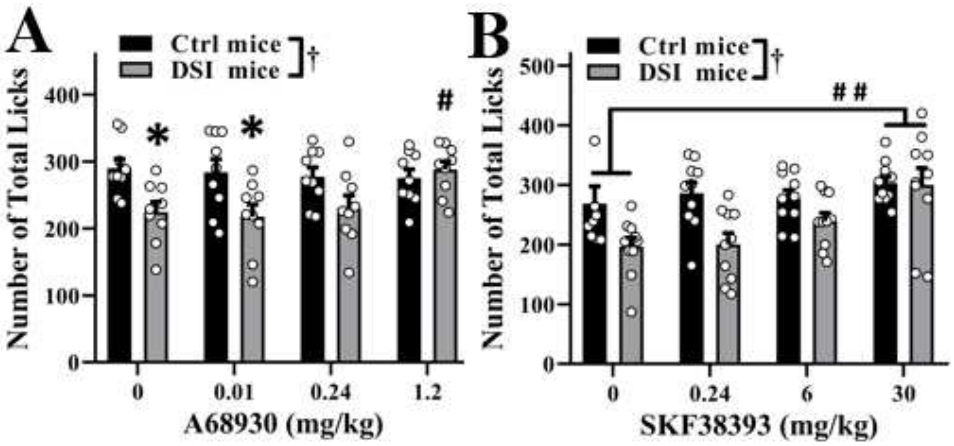

Fig. 7 Comparison of numbers of total licks after various doses of A68930 **(A)** or SKF38393 **(B)** treatments. RM-ANOVA: †p < 0.05 for comparison of genotype; *p < 0.05, compared to Ctrl mice; #p < 0.05, ##p < 0.01, compared to vehicle treatment

Two studies evaluating hedonic impact with sucrose solutions showed that the number of licks is a sensitive measure and reflects small changes in hedonic value (e.g., low sucrose concentrations) (Uematsu et al., 2011; Dastugue et al., 2018). The finding (Fig. 7) that D1 agonist treatment restored the number of licks but not the number of bursts in DSI mice supports this, suggesting that the recovered lick number largely reflects an increase in the hedonic impact. There is another possibility that D1-like receptors may be involved in mechanisms, such as postingestive feedback and thirst perception (Bouchaud & Bosler, 1986; Miyahara et al., 2012). D1 agonist treatment might affect those mechanisms and thus ameliorate the drinking behavior of DSI mice.

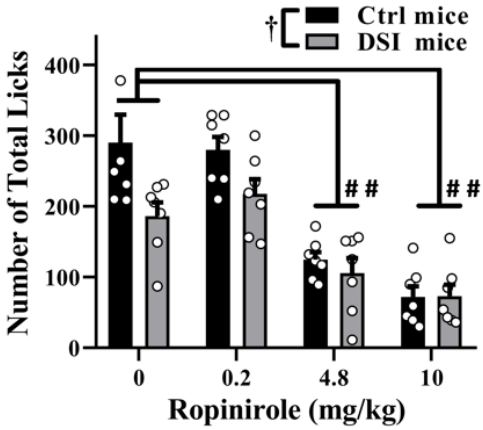

Fig. 8 Comparison of numbers of total licks after various doses of ropinirole treatments. RM-ANOVA: †p < 0.05 for comparison of genotype; ##p < 0.01, compared to vehicle treatment

D2-like receptor was reported to be critical for performing learned response (Randall et al., 2012; Lopez et al., 2015). In the present study, the D2/3 agonist ropinirole treatment (Fig. 8) decreased the intra-burst lick

speeds of both DSI and control mice, reflecting the involvement of D2-like receptors in movement (Gerfen, 1992). It also reduced the number and size of bursts, indicating lower incentive motivation and decreased hedonic impact during water drinking. A decrease in burst size after D2-like receptor antagonist treatment was also reported by previous studies (Schneider et al., 1990; Genn et al., 2003; Galistu & D'Aquila, 2013). Of note, both D2-like receptor agonist and antagonist were reported to suppress the performance of acquired conditioned responses (Lopez et al., 2015; Fraser et al., 2016). Accordingly, interfering with DA D2-like receptor signaling (by either stimulation or blockade) seems to suppress fluid ingestion, but the underlying cause may not be straightforward and may involve several neural mechanisms.

Chapter 3 : Dopamine D2-like Receptor Agonist Changes Effort-based Decision-making of Mice

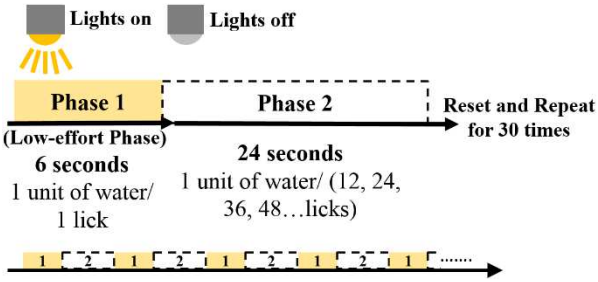

Fig. 9 Effort-based choice task based on licking test.

DA depletion or antagonism promotes a low-effort bias when choices between pressing a lever for preferred food and consuming currently available, ordinary chow are given (Salamone, Correa, Yang, Rotolo, & Presby, 2018). In the effort-based choice task of this study (Fig. 9), low-effort bias [ratio of phase 1 licks to phase 2 licks is bigger than time distribution (6 sec/24 sec = 0.25)] was equally observed in both control and DSI mice. The low-effort bias was not affected by SKF38393 treatment, but it was prevented by ropinirole treatment regardless of genotype (Fig. 10). As a previous study suggested that D2 receptor is critical for a reward cue to be incentive (Fraser et al., 2016), the cues which indicate the low effort (light on and the sound of frequent water pumping) might fail to boost the licking during phase 1 because of the interference of D2 receptor signaling caused by ropinirole treatment. This result suggests that D2-like receptors may play an important role in the decision-making of mice.

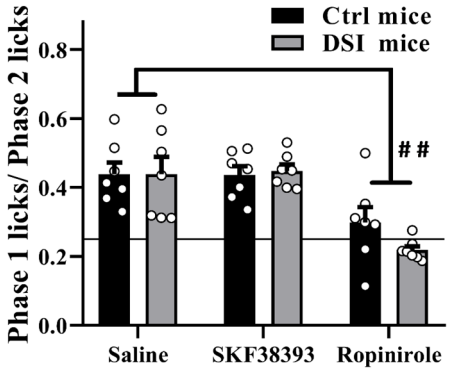

Fig. 10 The ratio of phase 1 licks to phase 2 licks after agonist administration. ##p < 0.01, compared to saline treatment

	Lick number	Intra-burst Lick speed	Burst size	Burst number
DSI mice	↓	→	→	↓
DSI mice + D1 agonist	→	→	→	↓
DSI mice + D2/3 agonist	↓↓	↓	↓	↓↓

Table 1. Summary of licking microstructures after DA agonist administration.

Conclusion:

Studies of reward-oriented behavior contribute to our understanding of how animals modulate their behaviors in response to environmental changes and their needs. In this study, triple transgenic (DSI) mice were developed to help elucidate the roles of DA in reward-oriented behavior by analyzing water drinking behavior. Specifically, the DSI mouse model enabled investigations of impact of DA deficiency and restoration of DA signaling. The findings may contribute to new treatments for illnesses related to DA loss, including anorexia nervosa, as suggested by G. K. W. Frank (2014). This study reveals that D1 agonist A68930 ameliorates the suppression of water drinking resulting from DA loss (Table 1), whereas the D2/3 agonist ropinirole impedes water drinking and prevents low-effort bias regardless of DA status. The findings may suggest the involvement of DAergic neurons in mechanisms, which are related to postingestive feedback and thirst perception, in addition to the other ones (motor control, hedonic impact of reward and incentive motivation), and indicating the importance of D2-like receptors in water drinking behavior and decision-making of mice.