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）

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ROLE OF DOPAMINE IN REWARD-ORIENTED BEHAVIOR：WATER DRINKING BEHAVIOR IN MICE WITH REDUCED DOPAMINE SECRETION

By

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A Dissertation

Presented to the faculty of

Department of Integrated Biosciences

In partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Graduate School of Frontier Science

The University of Tokyo

February 2020

Contents

Abbreviation

ANOVA	:	analysis of variance
CS	:	conditioned stimulus
Ctrl	:	control
DA	:	dopamine
DAergic	:	dopaminergic
DAPI	:	4’,6-diamidino-2-phenylindole
DSI	:	dopamine secretion interference
EYFP	:	enhanced yellow fluorescent protein
GABA	:	gamma-aminobutyric acid
L-DOPA	:	L-3,4-dihydroxy-phenyalanine
MSN	:	medium spiny neuron
NAc	:	nucleus accumbens
PCR	:	polymerase chain reaction
RM-ANOVA	:	repeated-measures analysis of variance
SEM	:	standard error of the mean
SN	:	substantia nigra
TBS-X	:	0.1% Triton X-100 in Tris-buffered saline
tetX	:	tetanus toxin
TH	:	tyrosine hydroxylase
tTA	:	tetracycline transactivator
VTA	:	ventral tegmental area

Abstract

動物における行動の多くは報酬あるいはその予測により誘導され、恒常的調節(ホメオスタシス)により修正を受ける(Mangel & Clark, 1986; McNamara & Houston, 1986)。そのため、動物の報酬志向行動は外的な報酬の変化、空腹や水分不足などの体内状態の変化によって常に影響を受けている(Bindra, 1978; F. M. Toates, 1986)。このような行動調節の仕組みを解明することは、判断決定の機構を理解するために不可欠である。これまでの研究から、中脳辺縁系ドーパミンと報酬志向行動の関連性が示唆されている(Berridge, 2018; Flagel & Robinson, 2017)。薬理研究の結果から、側坐核に対するドーパミンニューロンを介した刺激は、衝動消滅訓練を受けたラットに対して、報酬を求める衝動を再び起こすことができる(Peciña & Berridge, 2013)。Figure I に示すように、ドーパミン神経の投射は、大別して VTA や RrF から NAc へ至る経路と、SNc から striatum へ至る経路の二種類がある。報酬志向行動に関しては、前者である VTA や RrF から NAc へ至る経路に関係した研究が多く行われてきているのに対し、後者に関しての研究はあまり多くはない。

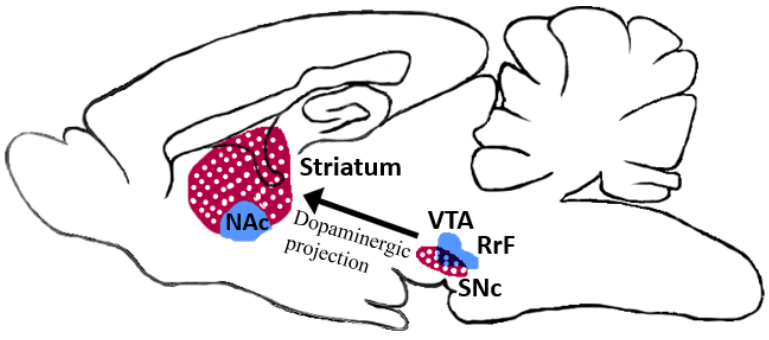

Figure I マウス脳におけるドーパミン神経の分布と投射方向。VTA, ventral tegmental area; RrF, retrorubral field; SNc, substantia nigra pars compacta; NAc, nucleus accumbens。

行動調節の仕組みを明らかにするため、齧歯動物の飲水行動を題材にして、wanting(誘因動機)や liking(報酬を得ることに対する喜び)の視点から研究が行われている(D'Aquila & Galistu, 2017; Dastugue, Merlin, Maquart, Bernard, & Besnard, 2018; Johnson, 2018)。なお本研究において、wanting(誘因動機)とはマウスが好ましい報酬である水に対して近づきそして摂取しようとする動機（積極性）を表し、liking(報酬への喜び)とは水報酬を得た後感覚系によってもたらされる hedonic impact（喜びの効果）を表す。動物は外部刺激（多様な味覚、食物のさまざまなテクスチャなど）および内部状態（空腹感と渇き）に従って報酬志向の行動を絶えず変更するため、外部刺激と内部状態の両方が強化できる強力な要因となる。そして、ドーパミン受容体は齧歯動物の飲水行動を調節することが報告されている。ドーパミン受容体はその性質から D1 様受容体と D2 様受容体の二つに大別される。そして、D1 様受容体と D2 様受容体は各々に誘因動機("wanting")と報酬の喜び効果("liking")へ影響を与えている可能性が示唆された(Galistu & D'Aquila, 2013; Genn, Higgs, & Cooper, 2003; Robles & Johnson, 2017)。

本研究では、行動調節におけるドーパミンの役割を解明するために、新たなトランスジェニックマウスを作製し、マウスの licking 行動を微細に分析する(number of licks, number of bursts, size of bursts, intra-burst lick speed を計測する)ことを通じて、ドーパミン分泌障害による行動変化を調べた。新種のマウスはドーパミン神経からのドーパミン分泌の一部が障害されると予想されたため、ドーパミン分泌障害マウス(DA secretion interference : DSI mouse)と呼ぶことにした。この DSI マウスにおいては、ドーパミンの放出をほぼ完全に抑える Dopamine deficient(DD)マウス(Palmiter, 2008)とは異なり個体の生存のために L-DOPA を必要としないため、生理的条件に近い状態でドーパミンの欠乏(あるいは低下)の影響とドーパミン受容体シグナリング

による機能回復について研究をすることが可能になった。しかし、障害されたドーパミンニューロンの数は全体の約半分ぐらいなので、対照群マウスとの行動の差異が少なく、実験がやや困難であることが短所であった。飲水行動と判断過程への影響を更に詳しく調査するため、飲水行動実験に先立って DSI 群と対照群のマウスにドーパミンの D1 受容体アゴニスト(A68930 または SKF38393)および D2/3 受容体アゴニスト(ropinirole)をする実験をあわせて実施した。

Chapter 1：Behavioral Change Induced by Reduced Dopamine Secretion

本研究において、TetX トランスジェニックマウス(Camk2a-loxP-STOP-loxP tetracycline transactivator と tetO-tetanus toxin) (Nakashiba, Young, McHugh, Buhl, & Tonegawa, 2008) と Slc6a3(DAT)-icre/ ERT2 マウス(Schriever et al., 2017)を掛け合わせることにより、DSI マウスを作製した(**Fig. IIA**)。DSI マウスの Cre recombinase の転写は Slc6a3(別名 Dopamine Transpoter: DAT) promoter によって誘導される。ドーパミン神経細胞にのみ転写が生じる。Tamoxifen を投与すると、ドーパミン神経細胞の loxP-STOP-loxP カセットが取り除かれ、tetX light chain の発現が始まる。その結果として、ドーパミンの分泌が tetX により障害される(**Fig. IIB**)。

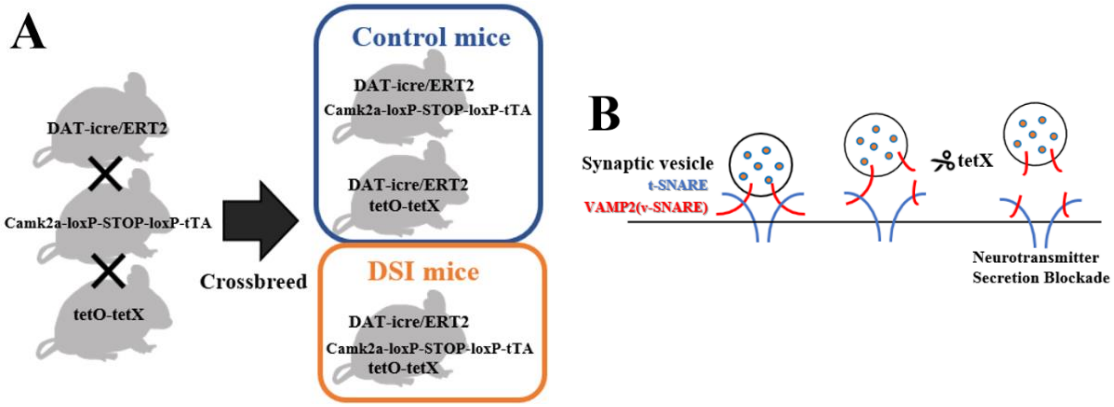

Figure II (A)DSIマウスを作製する育種図式。**(B)**tetXによる分泌障害(ドーパミン)。

Cre recombinase の発現の特異性を確認するために、DSI マウスと Gt(ROSA)26Sor$^{tm1(loxP-STOP-loxP-EYFP)Cos}$ マウスの掛け合わせを行い、得られたマウスを用いて免疫組織化学を実施した。その結果、Cre-loxP recombination により発現が誘導された EYFP 信号とドーパミン神経のマーカー信号(TH)が重なり合うことが示唆され(**Fig. III**)、これにより導入遺伝子の特異性が確認された。また、約全体半分のドーパミンニューロンでは Cre-loxP recombination が起きたことも確認された。

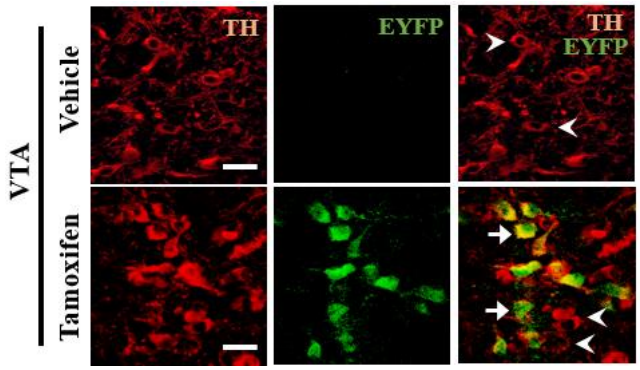

Figure III Creレポーター遺伝子の持つDSIマウスにおける TH(赤)とEYFP(緑)の二重染色。比例尺、25 μm。

DSI マウスのドーパミン生産量調べるために、マイクロダイアリシス実験を行った。その結果、tamoxifen 投与済みの DSI マウスでは対照群マウスと比較して脳内ドーパミン濃度の減少が認められた(**Fig. IVA**)。線条体では 61.4%、側坐核では 54.5%まで減少していることを確認した。DSI マウスは対照群マウスと同じ体重と平均飲水量であった。運動機能に関してはロータロッド試験で評価を行った。その結果、モーター制御による障害(**Fig. IVB**)(Sidak test, Ctrl vs. DSI; day 7, $p = 0.004$; day 8, $p = 0.021$) が厳しい条件下(回転速度 ≥ 28rpm)で起きていることを確認した。

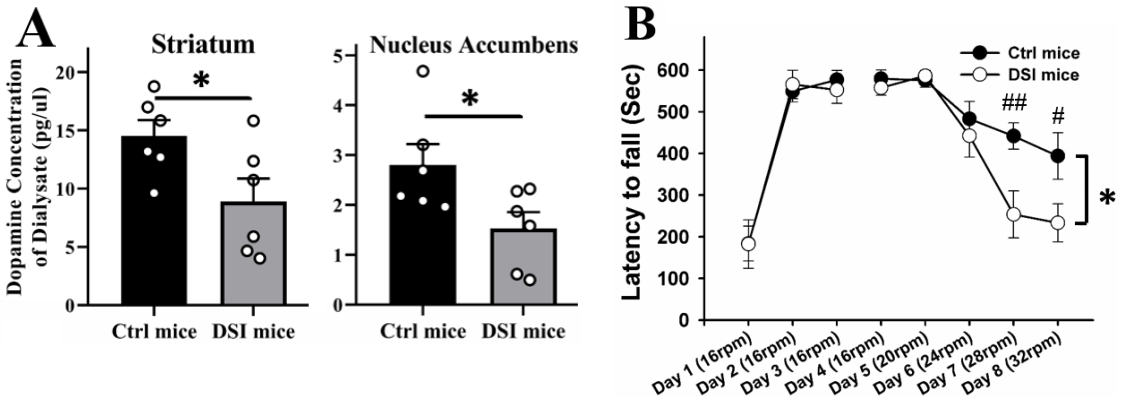

Figure IV (A)線条体と側坐核の透析液におけるドーパミン濃度。**(B)**ロータロッド試験。RM-ANOVA: *$p < 0.05$、対照群マウスとの比較。#$p < 0.05$と##$p < 0.01$、一日中の比較。

DSI マウスの学習記憶能力については、モリス水迷路の空間学習と逆転学習を用いて評価を行った。その結果、対照群マウスと比較し有意な差はなく、この評価法で調べる限り学習記憶能力は正常であった(Fig. V)。

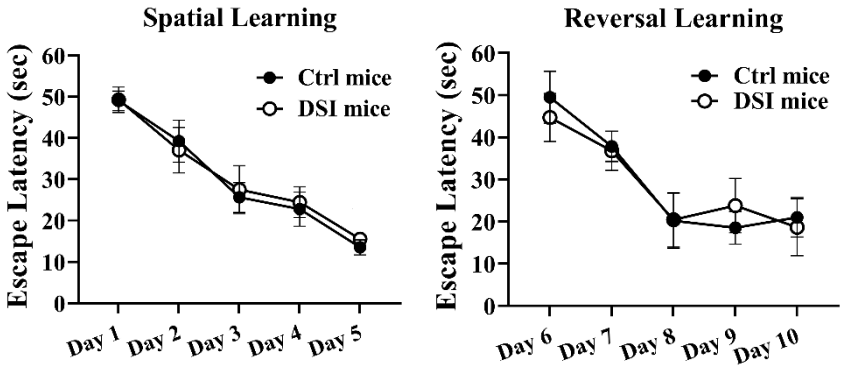

Figure V モリスウォーターメイズにおける空間学習と逆転学習。

飲水行動におけるリッキング回数(numbers of total licks)は行動への動機の強さ(欲求)を反映する。飲み物への摂取状況変化への指標として広く用いられている(Davis, 1989; Mendez, Maidment, & Murphy, 2016)。端的に言えば、リッキング回数が多いとは、飲水への欲求が強いことを示していると考えられる。しかし、先に述べたように、普通の状態では DSI マウスの飲水量は対照群マウスと比べて差はなかった。飲水量の制限という条件は、飲水行動に対する誘因を増強することができる。そこで、本研究では誘因増強状況において、ドーパミン分泌減少による飲水行動に対する影響の調査を行った。

7 日間の飲水行動試験においてマウスが水を摂取することを学んだ。この際、舐める動作によって水が得られることを学習させたのだが、ドーパミン分泌の減少のため、飲水制限下の DSI マウスでは舐める回数が有意に減少した(**Fig. VI**)。Numbers of licks は包括的な欲求の強さを表すと考えられ、DSI マウスでは舐める回数が減少していた。つまり、喉が渇いている状況において水に対する欲求が減少していると考えることができる。

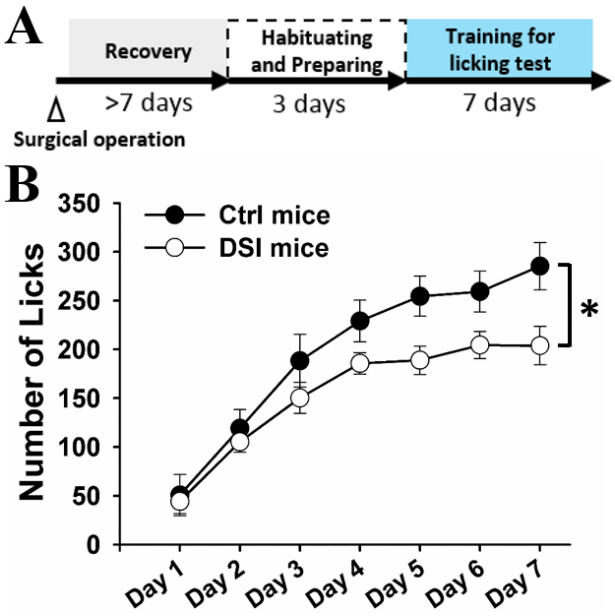

Figure VI (A)飲水行動訓練の図式。**(B)**訓練におけるリッキング回数
RM-ANOVA: *$p < 0.05$、対照群マウスとの比較。

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

齧歯動物の連続したリック動作(連続した二回以上のリックの間隔が 0.4 秒以内であること)を burst あるいは bout と呼称する(**Fig. VII**)(D'Aquila & Galistu, 2017; Johnson, 2018)。連続したリック動作の回数(number of bursts)は行動の活性化を、すなわち誘因動機(報酬に対する積極性)の強さを反映する。一方で、一回の連続したリック動作の長さ(size of the bursts)は報酬の喜び効果(味覚と食感の影響)の強さを反映する。また、連続したリック動作の速さ(intra-burst lick speed)は舐める動作におけるモーター制御(舌出し動作の敏捷性)の状態を示す。本研究では、飲水行動全体のリッキング回数と最初の一分間の burst(水分摂取後の影響が出る前に)を計測した。

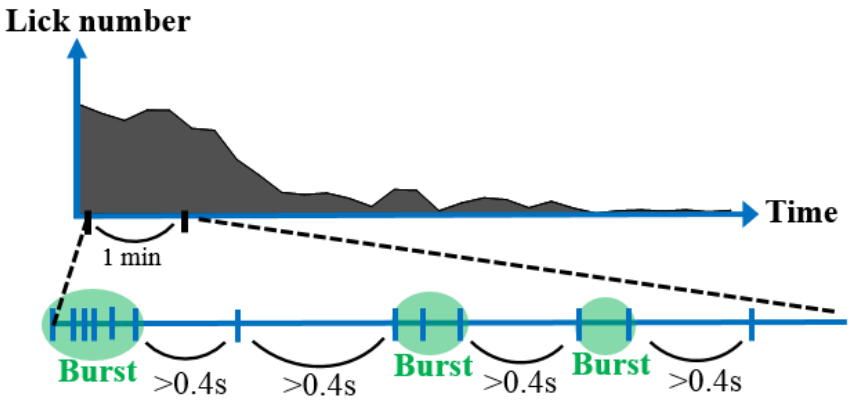

Figure VII リック動作における Burst に基づく Licking Microstructure(微細構造)を表す概念図。

ドーパミン D1 受容体アゴニスト(A68930)の投与は、減衰していた DSI マウスの飲水行動への欲求を回復する効果があった。**Fig. VIIIA** に示すように、本研究において初めて見出した知見である。さて、SKF38393 投与は対照群マウスの numbers of total licks まで増加させていた(**Fig. VIIIB**)。この A68930 と SKF38393 の効果の差異は、受容体アゴニストの脳内分布や受容体への応答

性の違いから生じると予想される。また、D1 受容体アゴニストの効果は神経への刺激により変化する可能性も考えられる。Numbers of total licks で評価される脳の活動は、わずかな喜び効果の変化に対する敏感性と関連している可能性がある(Dastugue et al., 2018; Uematsu et al., 2011)。従って、D1 受容体アゴニスト投与における結果は、喜び効果を増強することにより DSI マウスの飲水行動を回復できた可能性があげられる。また、D1 受容体が水を摂取後の渇きに対する充足の検知に関与している可能性も報告されている(Bouchaud & Bosler, 1986; Miyahara, Ono, Hitomi, Hirase, & Inenaga, 2012)。D1 受容体アゴニストの投与によって、飲水行動が回復した今回の結果は、これまでにあげられていた研究仮説を初めて具体的に証明した初めてのケースであると言うことができる。

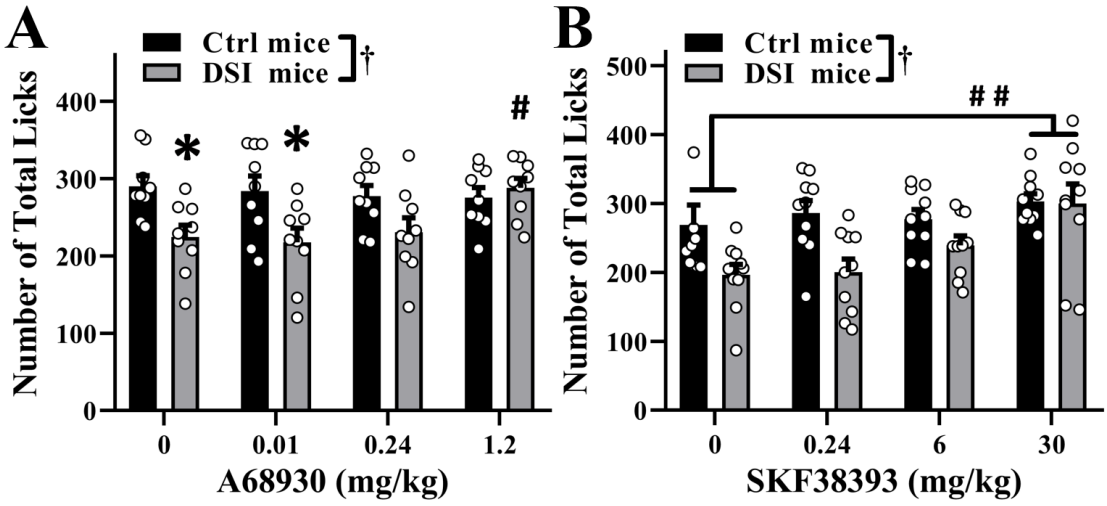

Figure VIII A68930**(A)**またはSKF38393**(B)**の投与後のnumbers of total licks比較。RM-ANOVA: †$p < 0.05$、遺伝子型の比較。*$p < 0.05$、対照群マウスとの比較。#$p < 0.05$と##$p < 0.01$、溶媒との比較。

D2 様受容体は学習した行動を再び行うことに不可欠だと報告されている(Lopez, Karlsson, & O'Donnell, 2015; Randall et al., 2012)。本研究においても、D2/3 受容体アゴニスト(ropinirole)の投与は、全てのマウスにおいて intra-burst lick speed を減少させた(**Fig. IX**)。また、モーター制御における D2 受容体の関与を反映した(Gerfen, 1992)number of bursts と size of

the bursts も減少させたことから、誘因動機と報酬の喜び効果を減少させたと推定された。D2 受容体アンタゴニスト投与による size of the bursts の減少は、他の研究においても報告されている(Galistu & D'Aquila, 2013; Genn et al., 2003; Schneider, Davis, Watson, & Smith, 1990)。また、D2 様受容体のアゴニストおよびアンタゴニスト投与は学習した行動を抑制する効果があった(Fraser, Haight, Gardner, & Flagel, 2016; Lopez et al., 2015)。以上により、ドーパミン D2 様受容体のシグナリングを刺激または阻害して撹乱することにより、飲水行動が抑制されたと考えられる。しかし、その変化はいくつもの神経回路に関与しており、非常に複雑であるとも考えられる。

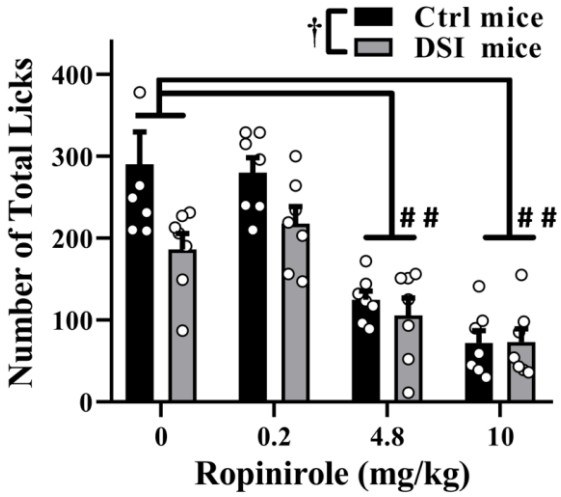

Figure IX ropiniroleの投与後のnumbers of total licks比較。RM-ANOVA: †$p < 0.05$、遺伝子型の比較。##$p < 0.01$、溶媒との比較。

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

動物は常に体力の支出と報酬の良さを評価して(天秤にかけて)行動を選ぶ。そのために、行動経済学の視点からドーパミンの役割を分析する研究もよくある。ドーパミン枯渇やアンタゴニストの投与は、二つの選択肢(レバーを

押して好物をもらうか、既にそばにある普通の餌を食べるか)がある場合に、低労力傾向(後者)を促進する(Salamone, Correa, Yang, Rotolo, & Presby, 2018)。本研究に採用した労力負荷による選択タスク(**Fig. X**)には、DSI マウスと対照マウスの両方でトレーニングによって低労力傾向[(phase1 licks/phase 2 licks) > (6 sec/24 sec = 0.25)]が増すことが示された。低労力傾向に関わると推定されるドーパミンの関与は、DSI マウスではドーパミン産生抑制が約半分であるため、影響を受けなかったと考えられる。

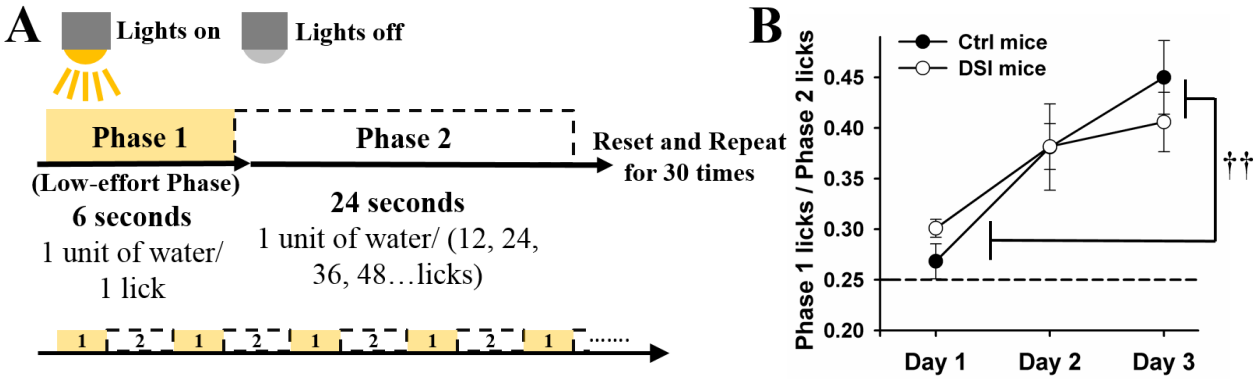

Figure X (A)労力負荷による選択タスク。**(B)**訓練におけるphase 1とphase 2の比率。RM-ANOVA: ††$p < 0.01$、Day 1との比較。

この低労力傾向は SKF38393 投与では影響が見受けられなかったが、ropinirole 投与においては阻害された(**Fig. XI**)。先行研究により D2 受容体は報酬合図(reward cue)による誘因動機を起こすために必要であることが報告されている(Fraser et al., 2016)。Ropinirole 投与によって phase 1 でのリッキング回数が相対的に低下した理由として、通常の条件で起こっていたと考えられる D2 様受容体を通じたドーパミン放出を通じた何らかの制御が、アゴニストによる過剰な D2 受容体刺激により消失したことが考えられる。この

仕組みを理解する上で、D2 アンタゴニストを使用した実験が有効であると思われるが、この結果は、D2 様受容体が状況によるマウスの判断過程に重要な役割を果たすことを示すこれまでの研究結果と一致している。

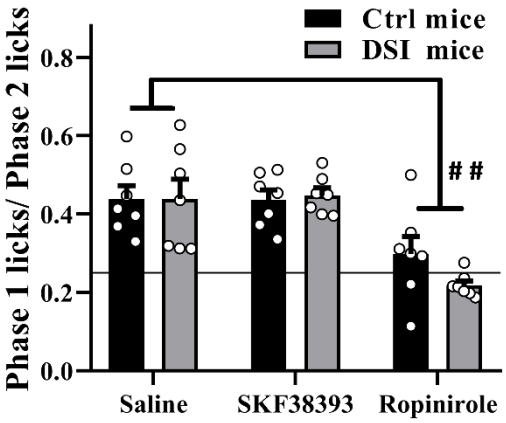

Figure XI 食塩水、D1アゴニスト(SKF38393)またはD2/3アゴニスト(ropinirole)の投与後のphase 1とphase 2の比率。RM-ANOVA: ##p < 0.01、食塩水投与との比較。

Conclusion：

本研究では、報酬志向行動におけるドーパミンの役割を調べるために、新しいタイプのトランスジェニックマウス(DSI)を用いた研究を実施した。この DSI マウスにおいては、ドーパミンの放出が通常マウスと比較して半分程度に抑制されており、報酬志向行動における２種類のドーパミン受容体(D1 タイプ・D2 タイプ)のはたらきを、それぞれのアゴニストを用いて調べることに適していた。これまでにドーパミンの放出をほぼ完全に抑える DD マウスを用いた研究が実施されてきた。この DD マウスの仕掛けは複雑であるため、仕組みの説明は review 論文に任せることにするが(Palmiter, 2008, p37-38)、ドーパミン合成に関わる酵素(Tyrosine Hydroxylase: TH)をノックアウトしたマウス(胎生１４日で死亡する)にノルエピネフリンニューロンでのみ TH 酵素を発現さ

せるようにすると生後１０日目までは正常に発生できるが、その後、生後２０日までには(摂食行動ができずに)死亡する。しかし、この DD マウスに、ドーパミンの前駆物質である L-DOPA を、１日に１回投与すると生存を維持できるようになる(Zhou & Palmiter, 1995)。L-DOPA 投与後のピーク時において、正常レベルの約 10%、ドーパミンが合成されていた。この複雑な条件下において、各種の実験が実施され、報酬行動におけるドーパミンの役割が、次々に明らかにされていった。

本研究においては、３種類の遺伝子コンストラクトを用いて正常レベルの約 50%、ドーパミンが合成できるマウス(DSI マウス)を作出することが出来た。この条件下において、飲水行動中の報酬志向行動におけるドーパミンの役割を解明した。この DSI マウスにおいては、DD マウスとは異なり個体の生存のために L-DOPA を必要としないため、生理的条件に近い状態でドーパミンの欠乏(あるいは低下)の影響とドーパミン受容体シグナリングによる機能回復について研究をすることが可能となった。

本研究では、D1 アゴニストがドーパミン分泌の低下により抑制状態にある飲水へのモティベーション(渇きに対する検知力または報酬の喜び効果)を回復させることを示した。一方で、D2/3 アゴニストは飲水行動へのモティベーション(誘因動機及び報酬の喜び効果)をさらに低下させた(Table I)。DD マウスの場合は、ドーパミン放出の量が極端に制限されているため、D2/3 アゴニストの投与によっても逆に飲水行動へのモティベーションが回復することが示されている。一般的に、臨床の現場などにおいて、D1 アゴニストは活性化の方へ働くのに対し、D2 アゴニストは抑制的に作用するとされており、DSI マウスで得られた知見は、臨床的な事柄と整合性が付いている(つじつまが合っている)。

以上の理由から、本研究で得られた成果(D1 アゴニストによるモティベー

ションの回復)は、ドーパミン不足による疾患(神経性無食欲症)の治療にも貢献できる(Frank, 2014)、と考えられる。また、モデル動物を用いた研究から、高齢になると脳内ドーパミン濃度が半分ほどになることも報告されている(Flood & Coleman, 1988; Friedemann & Gerhardt, 1992)。新しい種類の D1 アゴニストである A68930 は、ドーパミン分泌低下のために抑制された報酬志向行動(渇水時の飲水行動) が回復できることが示された(Table I)。ドーパミン作動薬に関して、臨床の現場においては、D1 作動薬が投薬されるケースは少なく、ropinirole など D2/3 アゴニストが汎用されているが、本研究において、D1 アゴニストの有用性について、新たな光を当てることができたと思われる。

	Lick number	Intra-burst Lick speed	Burst size	Burst number
機能の指標	欲求	モーター制御	喜び効果	誘因動機
DSI mice	↓	→	→	↓
DSI mice + D1 agonist	→	→	→	↓
DSI mice + D2/3 agonist	↓↓	↓	↓	↓↓

Table I ドーパミン受容体アゴニスト投与後のリック動作(微細構造)における４つの指標。

General Introduction

Dopamine transmission and reward-oriented behavior

Mesolimbic dopaminergic (DAergic) projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is important for cues to motivate animals to seek for reward (Fig. i) (Baldo & Kelley, 2007; Dickinson, Smith, & Mirenowicz, 2000; Halbout et al., 2019; Ostlund & Maidment, 2012). DAergic projections from the substantia nigra (SN) to the dorsal striatum also plays an essential role especially in terms of initiating behavior (Palmiter, 2008). Activation of mesolimbic dopamine (DA) neurons is thought to reinforce behaviors and endow predictive cues with the reinforcing property of reward (Halbout et al., 2019; Sharpe et al., 2017; Steinberg et al., 2014), and thus the cues may also become target of behaviors. Moreover, the degree to which DAergic neurons are phasically excited by rewards proportionally evokes subsequent cue-triggered DA response (McClure, Daw, & Montague, 2003; Schultz, Stauffer, & Lak, 2017).

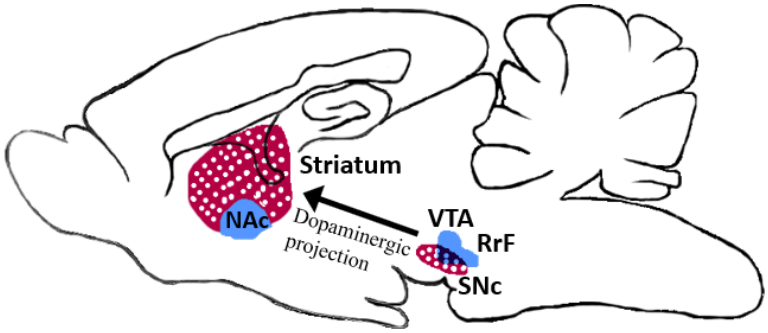

Figure i
Distribution of DAergic neurons and their projections in an adult mouse brain. VTA, ventral tegmental area; RrF, retrorubral field; SNc, substantia nigra pars compacta; NAc, nucleus accumbens.

While DA is known to play an important role in pairing a cue (conditioned stimulus, CS) with a reward, its role in the expression of previously learned responses is less clear. The responses to approach the learned locations of a lever (reward cue) or a reward are reported to be mediated differentially by the mesolimbic DA system (Flagel & Robinson, 2017). Flupenthixol, an antagonist of both DA D1 and D2 receptors, administration directly into the core of the NAc attenuates the contact with lever (sign-tracking) but not the contact with food-cup (goal-tracking) (Saunders & Robinson, 2012). A recent study also indicated that chemogenetic inhibition of VTA DAergic neurons disrupts the reinforcing property of CS to enhance reward seeking response (lever pressing), but the ability of CS to increase reward retrieval response (food-cup approach after lever pressing) was spared by that inhibition (Halbout et al., 2019). Their findings indicate that the CS-evoked response targeting a reward cue is highly dependent on VTA DAergic neurons while the response targeting a reward is not. Therefore, the VTA DAergic neurons likely contribute to the behaviors that comprise many steps and require more effort to complete, but they do not play a necessary role in the expression of learned responses. Consistent with this thought, extended training reduces the cue-evoked DA release and makes the learned response less dependent on DA transmission (Clark, Collins, Sanford, & Phillips, 2013; Levesque et al., 2007; Wassum, Ostlund, & Maidment, 2012).

There are less studies which examined the role of the nigrostriatal pathway (projection from the SN to the dorsal striatum) in facilitating behavior because complete lesions of this pathway result in severe motor control impairment (Gerfen, 1992; Puglisi-Allegra & Ventura, 2012). However, the DAergic projection from the SN may be as important as that from the VTA because neural activity in the nigrostriatal pathway specifically signals the initiation or the termination of actions

(Jin & Costa, 2010). The studies by R. D. Palmiter showed that restoring DA signaling selectively to the dorsal striatum by viral gene therapy in DA-deficient mice is sufficient to restore feeding behavior and reward-based learning (Palmiter, 2008). The rescued mice also expressed normal motivation to engage in reward-oriented behaviors (Darvas & Palmiter, 2009).

Previous studies of DA-deficient mouse

Taking advantage of gene targeting techniques, Q.-Y. Zhou and R. D. Palmiter generated a new knockout mouse line (Zhou & Palmiter, 1995) by inactivating the both *Tyrosine hydroxylase* (*Th*) alleles of mouse and introducing a *TH* gene under the control of the *Dopamine β-hydroxylase* (*Dbh*) locus (Thomas, Matsumoto, & Palmiter, 1995; Zhou, Quaife, & Palmiter, 1995). The $Th^{-/-}$, $Dbh^{Th/+}$ mice cannot produce DA anywhere except in noradrenergic neurons (Fig. ii), whose function is necessary for embryos to survive. Those mice are referred to as DA-deficient mice in this dissertation to distinguish them from the new transgenic (DSI) mice generated by our lab.

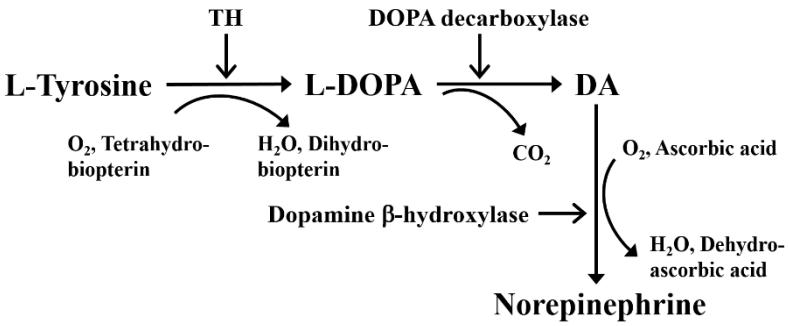

Fig. ii Catecholamine biosynthesis pathway.

DA-deficient mouse pups are essentially indistinguishable from control littermates during the first 10 days since birth, after which their body weights are gradually exceeded by those of control littermates (Zhou & Palmiter, 1995). The DA-deficient mice then become hypoactive and will decease because of insufficient

feeding unless the mice receive daily injection of L-3,4-dihydroxy-phenyalanine (L-DOPA) (Szczypka et al., 1999). After L-DOPA injection, the DA-deficient mice become active and begin to eat food until the level of DA returns to less than 1% of normal again. Although the DA system in DA-deficient mouse develops normally in the absence of DA production, the post-synaptic, medium spiny neurons (MSNs) show hypersensitivity to DAergic stimulation. For example, striatal c-fos expression can be induced by concentrations of DA or D1 receptor agonist (SKF81297) that have no effect in normal mice (Kim, Szczypka, & Palmiter, 2000), and the extracellular signal-regulated kinase is activated by D1 receptor agonist not only in the NAc but also in the dorsal striatum (Kim, Palmiter, Cummins, & Gerfen, 2006). Those reports suggest that DA-deficient mice response to DAergic stimulation slightly differently from normal mice, even though the adaptation of MSNs to lack of DA signaling can be reversed by semi-chronic L-DOPA treatment (5 times per day) (Kim et al., 2006).

Drugs such as amphetamine that facilitate DA release are shown to inhibit feeding of mice (Cannon, Abdallah, Tecott, During, & Palmiter, 2004). DA-deficient mice are spared from the inhibitory effect of amphetamine, but the feeding behavior of DA-deficient mice can be inhibited by amphetamine if the DAergic projection to the dorsal striatum has been restored by viral gene therapy (Cannon et al., 2004; Sotak, Hnasko, Robinson, Kremer, & Palmiter, 2005). Even without the DAergic projection to NAc and striatum, DA-deficient mice are able to learn the location of food; however, DAergic projection is necessary for demonstrating what they have learned (Robinson, Sandstrom, Denenberg, & Palmiter, 2005). It is unclear if the DA-deficient mice perceive and evaluate the food as the normal mice do.

In spite of the infirmity and the less food and water consumption compared to control littermates, the studies of DA-deficient mice by R. D. Palmiter and his

colleagues indicate the importance of DA signaling within the striatum in motivation, suggesting that the nigrostriatal pathway alone is sufficient to allow feeding, locomotion, and reward-based learning (Darvas & Palmiter, 2009; Palmiter, 2008).

Taste supports the reward-oriented behaviors in eating and drinking

Animals can modulate their behaviors in response to environmental changes and their needs. However, the precise mechanisms underlying this behavioral modulation remain unclear. One of the crucial abilities is to distinguish 'acceptable' foods from the potential 'toxic' foods (Breslin, 2013; Mennella, Daniels, & Reiter, 2017). This ability is important for surviving in an environment where nutrients are scarce, and it has been manipulated by evolution over millions of years. We perceive the taste of food after chewing and dissolving it into saliva. The taste percepts are elicited by stimulating the taste buds located in the oral and pharyngeal epithelia with the molecules released from what we ingested. Taste system, which combines olfaction and oral somatosensation to form flavors, enables us to evaluate food and water and prepare the body for what we eat and drink (Dotson, Geraedts, & Munger, 2013; Zimmerman et al., 2016).

These sensations have been involved in reward system by evolution to perceive the environment, and animals use it to learn the physiological outcomes of ingestion and modulate their behaviors according to different tastes. If the taste meets the requirement for survival, it will bring strong hedonic impact to animal through orosensory mechanisms and encourage the animal to seek for the same taste. For example, intense saltiness that is disgusting and induces rats to express mouth gapes, headshakes, and arm flails can become attractive to rats under sodium appetite (Robinson & Berridge, 2013). Normally, rats that have learned a lever conditioned

stimulus, which predicts intense salt taste in their mouth, escape from the lever whenever it appears. However, the lever conditioned stimulus suddenly becomes wanted, once the rats are in a salt appetite state of sodium depletion. It is of note that the internal state (hunger or thirst) is a potent factor to reinforce behavior, but reducing hunger or thirst alone is usually not enough to motivate animal. For example, rats scarcely press lever to deliver nutrients directly to their stomach through a gastric fistula (Nicolaidis & Rowland, 1975) unless a mouthful of saccharin is given at the same time (Holman, 1969).

Objective 'liking' and 'wanting' during ingestion

Feelings of animals have long been of wide interest mainly because animals express their thought in their own ways. Contemporary psychologists and affective neuroscientists tried to resolve this puzzle by manipulating neural transmission in the brain (Berridge, 2018). Although it is hard to draw absolute distinctions, they used the words 'liking' and 'wanting' (with quotation marks), which have objective consequences and features that can be detected in physiology and/or behavior, to distinguish them from the accompanying conscious feelings liking and wanting, which are subjective and implicit.

To study the neural systems responsible for the hedonic impact, many studies have exploited the facial expressions of animal (Berridge et al., 2009), which includes newborn human infants, orangutans, chimpanzees, and rats, to measure the objective 'liking' reactions to sweet taste rewards. Specific sites in the limbic structures have been called 'hedonic hotspots' because opioid stimulation within those spots doubles or triples the number of 'liking' reactions elicited by sucrose taste (Berridge et al., 2009). However, opioid stimulation outside those hotspots can still stimulate 'wanting'

for food but do not enhance 'liking'. Therefore, 'liking' and 'wanting' rewards are anatomically dissociable.

Analysis of licking microstructure in drinking behavior

Central pattern generator is a group of neurons that generates repetitive patterns of motor behavior. The neural control of eating and drinking can be reduced to the modulation of the corresponding central pattern generators (Fay & Norgren, 1997; Travers, Dinardo, & Karimnamazi, 1997). Analysis of licking microstructure is used to reveal the modulation of rhythmic licking pattern during fluid ingestion (Fig. iii).

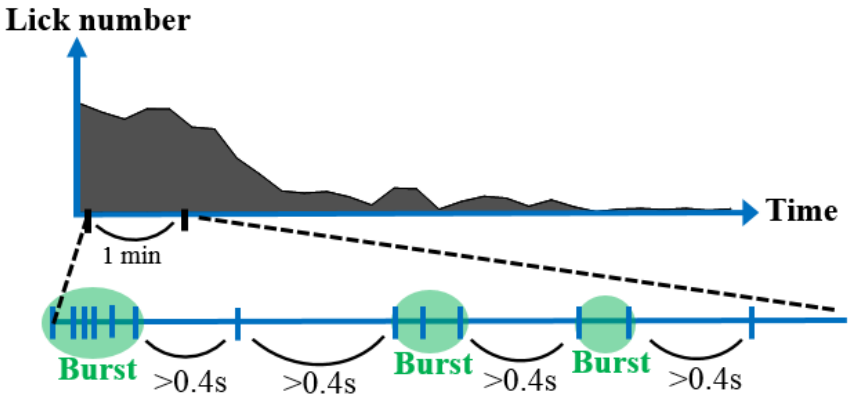

Fig. iii Scheme of the licking microstructure.

The study by J. Davis (1992) found that the size of burst increased, when the sucrose concentration was elevated, while the number of bursts showed an inverted U-shape curve. Therefore, the size and number of bursts were regulated differently. The findings also showed that blocking the entry of sweet solution into stomach, known as sham-feeding, significantly increased the number of bursts due to the lack of post-ingestive negative feedback. By contrast, the size of bursts was not affected by sham-feeding but increased by the elevated sucrose concentration (positive feedback). Therefore, within the framework of incentive salience attribution hypothesis (D'Aquila & Galistu, 2017; Johnson, 2018), the size of bursts is considered to be

influenced by the hedonic impact brought by the taste, and the number of bursts shows the degree of desire to consume liquid solution. The intra-burst lick speed is used as an indicator of licking-associated motor control to reveal the effect on tongue movement.

The aim is to reveal the roles of DAergic neurons in water drinking behavior

In this study, I described the measurable, mesolimbic form of wanting ('wanting') as incentive motivation, which is an immediate desire triggered by reward cues that promotes mice to approach and consume water reward. By contrast, 'liking' was revealed by the hedonic impact brought by the taste on the size of bursts during water drinking. Animals constantly alter their reward-oriented behaviors when the value of a cue or reward (taste and its corresponding experience) changes or when the internal state (hunger and thirst) shifts (Bindra, 1978; F. M.Toates, 1986). Elucidating this process of behavioral change is fundamental to understanding how a decision is made. Considerable evidence has indicated the involvement of mesolimbic DA system in reward-oriented behaviors (Berridge, 2018; Flagel & Robinson, 2017). Pharmacology studies showed that DAergic stimulation of the nucleus accumbens (NAc) triggers an intense response to obtain a reward, even if a rat has undergone extinction training (Peciña & Berridge, 2013). 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 (Galistu & D'Aquila, 2013; Genn et al., 2003; Robles & Johnson, 2017).

The experiments in chapter 1 were focused on generating a new transgenic mouse line with reduced DA secretion (DSI mouse) and revealed the influence of DA loss on the motor control and the water drinking behavior of mice. In chapter 2, the roles of DAergic neurons and DA receptors in reward-oriented behavior were

investigated from the perspectives of 'liking' (hedonic impact of reward) and 'wanting' (incentive motivation) by the licking microstructure analysis. Mice also received a subcutaneous injection of DA D1 receptor agonist (SKF38393 or A68930) or DA D2/D3 receptor agonist (ropinirole) to compensate for the moderate DA loss. Chapter 3 investigated the influence of DA loss on the effort-based decision-making of mice by a novel effort-based choice task based on licking test, which may provide new information about how a decision is made. The findings from this study further the understanding of reward-oriented behavior in fluid ingestion of mice and may contribute to new treatments for illnesses related to moderate DA loss.

Chapter 1

Behavioral Change Induced by Reduced Dopamine Secretion

Introduction

Most animal behaviors are driven by rewards. In the 1930s, B. F. Skinner developed a new method to study these behaviors and called it operant behaviors (Skinner, 1938). Operant behavior is said to meet two conditions: (1) It is spontaneously emitted by an animal, and there is no obvious triggering stimulus. (2) Its frequency or degree of activity can be enhanced or suppressed by consequences of behavior (F. Toates, 2012). Animals constantly alter their behaviors in response to homeostatic regulation (hunger and thirst) (Mangel & Clark, 1986; McNamara & Houston, 1986) and reward cues (conditioned and unconditioned stimuli) (Bindra, 1978; F. M.Toates, 1986), and thus operant behavior is sometimes called reward-oriented behavior to emphasize its variability (Goltstein, Reinert, Glas, Bonhoeffer, & Hübener, 2018; Tsutsui-Kimura et al., 2017). For example, when a corresponding reward cue appears, animals that are hungry or thirsty work more intensively to acquire a desired reward (food or water) than animals with *ad libitum* access to food and water (Campbell, 1960; Petrovich, 2011; Weingarten, 1983). That is, reward-oriented behavior changes as the animal's internal state fluctuates or the value of a cue or reward is revised. Elucidating this process of behavioral change is fundamental to understanding how a decision is made by an animal under different situations.

Considerable evidence has indicated the involvement of mesolimbic DA system in reward-oriented behaviors (Berridge, 2018; Flagel & Robinson, 2017), and DA antagonism changes the ingestive behaviors of rodents (Galistu & D'Aquila, 2013; Robles & Johnson, 2017; Salamone et al., 2018). Pharmacology studies showed that DAergic stimulation of the NAc triggers an intense response to obtain a reward, even if a rat has undergone extinction training (Saunders & Robinson, 2012; Peciña &

Berridge, 2013; Singer *et al.*, 2016). By contrast, direct administration of flupenthixol, a nonspecific DA receptor antagonist, to the NAc impairs responding for a reward (Danna & Elmer, 2010; Saunders & Robinson, 2012). Fluid ingestion of rodents has been used as a means to explore how 'liking' (hedonic impact of reward), 'wanting' (incentive motivation), and learning (reward prediction) modulate animal behaviors (Berridge, 1996; Berridge et al., 2009; D'Aquila & Galistu, 2017; Dastugue et al., 2018; Davies et al., 2015; Johnson, 2018; Taha & Fields, 2005; Uematsu et al., 2011).

Although it is contrary to a common view that DA signaling in NAc is involved in motivation to engage in reward-oriented behaviors (Baldo & Kelley, 2007; Salamone & Correa, 2002), previous studies with a DA-deficient mouse developed by R. D. Palmiter and his colleagues (Zhou & Palmiter, 1995; Palmiter, 2008) showed that DA signaling in the dorsolateral striatum was sufficient to serve these functions (Darvas & Palmiter, 2009). DA-deficient mouse exhibited infirmity and consumed less food and water due to loss of DA production (Palmiter, 2008). In order to avoid the drawbacks caused by severe DA loss, a tetanus toxin (tetX)-based method developed by S. Tonegawa that inhibits synaptic transmission (Nakashiba et al., 2008; Schiavo et al., 1992; Schoch et al., 2001; Yamamoto et al., 2003) was adopted to generate a transgenic mouse line with moderate inhibition of DA signaling (Kao & Hisatsune, 2019). Specifically, tetX transgenic mouse line (*Camk2a*-loxP-STOP-loxP- tetracycline transactivator [tTA] and *tetO*-tetX) was crossbred with a mouse line carrying the *Slc6a3*(DAT)-icre/ERT2 transgene (Gore et al., 2017; Schriever et al., 2017) that encodes a tamoxifen-inducible Cre recombinase under the control of the *Slc6a3* promoter, which is active specifically in DAergic neurons. The expression of tetX light chain in these mice is indirectly regulated by an α-CaMKII promoter, whose activity is relatively weak in DAergic neurons (Burgin *et al.*, 1990; Wang *et al.*, 2013). After

tamoxifen administration, the DAergic neurons whose loxP-stop-loxP cassette has been removed by Cre-loxP recombination begin to express tetX light chain and reduce their DA secretion. This new triple transgenic mouse line is expected to exhibit partial blockade of synaptic DA release rather than severely impaired DA secretion and thus was named the DA secretion interference (DSI) mouse line.

The DSI mouse line enables the study of phenotypes related to DA loss and the role of DAergic neurons and DA receptors in reward-oriented behavior. It may also contribute to new treatments for illnesses related to DA loss. In this chapter, the reduction of DA concentration in the brain of DSI mice was examined by microdialysis and immunochemistry. In addition, possible influences of suppressed DA secretion on motor control, limb strength and learning were investigated by rotarod test, grip strength test and Morris water maze respectively. Water drinking behavior of DSI mice under thirsty condition was compared with control mice by recording the total number of licks, which represents the degree of feeding activity.

Materials and Methods

Animals and drug treatment

C57BL/6-Tg(*Camk2a*-tTA)1Stl/J mice and C57BL/6-Tg(tetO-GFP/tetX)5696Stl/J mice (kindly provided by T. McHugh and S. Tonegawa) were crossbred with C57BL/6-Tg(*Slc6a3*-icre/ERT2)2Gloss/J mice purchased from Jackson Laboratory (Bar Harbor, ME, USA) to generate the DSI mice (Fig. 1.1A, B). To confirm the cell type specificity of Cre expression by immunohistochemistry, some DSI mice were further crossbred with B6.129X1-Gt(ROSA)26Sor$^{tm1(EYFP)Cos}$/J mice purchased from Jackson Laboratory. The DSI mice without *Camk2a*-loxP-STOP-loxP-tTA (Tg2) or tetO-tetX (Tg3) were used as control (Ctrl) mice. Three to four mice per cage maintained under a 12 h:12 h light-dark cycle (lights on at 08:00 a.m.) at 22°C were given *ad libitum* access to food and water. All animal procedures and experiments in this study were approved by the Ethical Committee of the University of Tokyo and were conducted according to the Guidelines for Animal Experimentation required by the University of Tokyo.

Tamoxifen (350 mg/kg, dissolved in corn oil) was orally administered to control and DSI mice (male, littermates, 12 weeks old) for 4 consecutive days, and the last administration was given at least 1 month before the behavioral experiments.

Mouse genotyping

Mouse tail biopsies are obtained from mouse pups between 21 and 28 days of age, and then incubated with tail lysis buffer (100 mM Tris-HCl, 200 mM NaCl, 5 mM EDTA, 0.5% Polyoxyethylene-20, pH 8.5; 5 μl/ml Proteinase K, Takara Bio Inc., code no. 9034) overnight at 52°C. Tail sample (in lysis buffer) was mixed with equal

volume of phenol/chloroform/iso-amyl alcohol (25:24:1) and then centrifuged at top speed for 5 min. The upper phase of the samples was retrieved and mixed with equal volume of chloroform and then centrifuged at top speed for 5 min. After retrieving the upper phase, the tail sample was mixed with 3 M sodium acetate in 100% ethanol in the ratio 1:2.5 to form DNA precipitate. The DNA precipitate was rinsed with 70% ethanol (-20 °C) and then dissolved in Tris-EDTA Buffer.

For identification of mouse genotype, 1 μl of DNA sample retrieved from each mouse tail biopsy was used in a subsequent polymerase chain reaction (PCR) with Taq PCR kit (code no. R007A) purchased from Takara Bio Inc. The final reaction mixture contained 25 mM TAPS, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM DTT, 200 μM of each dATP·dGTP·dCTP, 100 μM [^{3}H]-dTTP, 1 μM of forward and reverse primer, 1.25 U Taq DNA polymerase, and 0.2 mg/ml DNA sample. The DNA primer sets used in the PCR were as follow: Tg1 forward primer TGGCTTGCAGGTACAGGAGG, reverse primer AGACTTCCTCGGGCTCCCG; Tg2 forward primer CGCTGTGGGGC-ATTTTACTTTAG, reverse primer GGGTCCATGGTGATACAAGG; Tg3 forward primer AAGTTCATCTGCACCACCG, reverse primer TCCTTGAAGAAGATGG-TGCG. The PCR started with denaturation at 94°C for 1 min and followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min, and there was a final extension at 72°C for 2 min. PCR products were separated on a 1.5% agarose gel by electrophoresis and then stained with ethidium bromide.

Immunohistochemistry

Six weeks after tamoxifen administration, mice were anesthetized with a xylazine hydrochloride-ketamine hydrochloride solution (10 mg/kg body weight, 80 mg/kg;

intraperitoneally). After introducing a cannula into the ascending aorta through the left ventricle, the mice were transcardially perfused with phosphate-buffered saline and then with 4% formaldehyde. Brain samples were postfixed in 4% formaldehyde for 24 h and then incubated in 30% sucrose in phosphate-buffered saline for 2 days before being embedded in OCT compound (Sakura Finetek, Japan) and frozen at –80°C. The frozen brain samples were coronally sliced into 30 μm-thick sections on a cryostat (Microm, Germany) while kept at –20°C and submerged in cryoprotectant solution for preservation at –30°C.

For immunohistochemistry, the brain slices were rinsed with 0.1% Triton X-100 in Tris-buffered saline (TBS-X) three times and then incubated in 0.1% TBS-X containing 3% normal donkey serum for 30 min. For the staining of TH, which is a marker of DAergic neurons, and enhanced yellow fluorescent protein (EYFP), the samples were incubated with primary antibodies (mouse anti-TH IgG, 1:200, MAB318 [Millipore]; rabbit anti-GFP IgG, 1:500, 598 [MBL]) diluted in 0.1% TBS-X containing 3% normal donkey serum for 3 days at 4°C. After rinsing three times with 0.1% TBS-X, the samples were incubated with secondary antibodies (Alexa 647-conjugated anti-mouse donkey IgG, 1:1,000, A31571 [Invitrogen]; Alexa 488-conjugated anti-rabbit donkey IgG, 1:1,000, A21206 [Invitrogen]) for 2 h at room temperature in the dark. The samples were rinsed with TBS for 15 min and then incubated in 0.1% TBS-X containing DAPI (4′,6-diamidino-2-phenylindole; 1:5,000 [Sigma]) for 5 min. After rinsing with TBS to remove excess DAPI, the samples were mounted on glass slides and observed with a confocal microscope (TCS SP2; Leica, Germany). Images of the ventral tegmental area (VTA) or the substantia nigra (SN) were acquired at ×10 and ×40 magnifications, and the numbers of TH^+ and $TH^+/EYFP^+$ cells in four random 10,000 μm^2 areas in each image were calculated using ImageJ software (National Institutes of

Health, USA). The optical density of the TH signal within the VTA or SN was calculated by dividing the signal intensity by the signal area covering the cell bodies.

Microdialysis and analysis of the dialysate

Mice were anesthetized with a xylazine hydrochloride-ketamine hydrochloride solution (0.8 mg/kg, 8 mg/kg; subcutaneous) and placed in a David-Kopf stereotaxic apparatus. A microdialysis guide cannula was implanted above either the NAc (1.2 mm anterior and 1 mm lateral to bregma, 3.1 mm below the dura) or the striatum (1 mm anterior and 1.7 mm lateral to bregma, 2.0 mm below the dura). The guide cannula along with a stainless-steel screw anchored to the skull were secured by cranioplastic cement.

After 4 to 7 days of recovery from the surgery, a concentric microdialysis probe (0.20 mm inner diameter, 0.22 mm outer diameter, 50 kDa molecular weight cutoff, artificial cellulose membrane; Eicom) was inserted into the target area through the guide cannula (the probe extended 0.5 mm or 2 mm beyond the end of the guide cannula into the NAc or striatum, respectively). Microdialysis was conducted in the same plastic cage in which the mouse lived, and the mouse could move freely during the experiment. Sampling was performed after Ringer's solution (147 mM Na^+, 4 mM K^+, 2.3 mM Ca^{2+}, and 155.6 mM Cl^-) was circulated through the probe for 40 min at a flow rate of 1.2 μl/min (ESP-64 syringe pump; Eicom).

The obtained dialysates were manually injected into a high-pressure liquid chromatography system with a 10 μl Hamilton syringe and analyzed by electrochemical detection. The mobile phase consisted of 80% 0.1 M sodium phosphate buffer (pH 6.0) containing 20% methanol, 50 mg/liter EDTA-2Na, and 500 mg/liter 1-octanesulfonic acid sodium salt. DA was separated on a reverse-phase analytical column (Eicompak CA-5 ODS, 5 μm particle size, 2.1 mm inner diameter, 150 mm;

Eicom) and detected with an electrochemical detection system (ECD-700; Eicom) consisting of a graphite electrode set at +450 mV against an Ag/AgCl reference electrode (RE-500; Eicom).

Rotarod test and grip strength test

The motor control of mice was analyzed using a rotarod test. Before the training session, the mice were habituated by placing them on a rod for 10 min per day (0 rpm) for 3 consecutive days. During the 3-day training session, the mice were placed on the rod rotating at a steady speed (16 rpm) for 10 min. If a mouse fell off the rotating rod, it was immediately placed back onto the rod until the 10 min trial was over, and the time of the first fall was recorded as the latency to fall. On the next 5 days of testing, the rotation speed was increased 4 rpm per day from 16 to 32 rpm. The mice underwent three trials every day and were not placed back onto the rotating rod after falling. The latency to fall of each mouse in the testing session was calculated as the mean from the three trials.

A grip strength meter (MK-380M; Muromachi Kikai, Tokyo, Japan) comprising a metal grid attached to a force meter was used to evaluate the muscle force of the four limbs for each mouse. In every test session, a mouse was lifted by its tail to the height where the mouse grabbed the grid with all four limbs. After visually checking the tight grip with paws, the operator gently pulled the mouse away from the grid until the pulling force overcame the grip strength. The force meter recorded the peak pull-force achieved by the mouse. Each mouse was subjected to three consecutive measurements with 1 min intervals, and the mean value of the three measurements was recorded as the mouse's four-limb strength. Both tests were conducted by the same operator.

Morris water maze

Mice between 15 and 17 weeks of age were transferred and habituated to a dimly lit test room for 30 min prior to the experiment. A circular pool (120 cm in diameter) filled with opaque water made with white paint at 20°C was placed in the test room, and four large objects were hung near the pool as cues. The mice were trained to find a hidden circular platform (10 cm in diameter) placed in a predetermined quadrant (NE for spatial learning and SW for reversal learning) 1 cm below the water surface. During training for spatial learning (day 1 to day 5) and reversal learning (day 6 to day 10), the mice were released from four pseudorandomly assigned start locations (NW, NE, SW, and SE) and allowed to swim for 60 s. If a mouse did not find the hidden platform, it was manually guided to the platform and allowed to rest on the platform for 15 s. The mice underwent 6 training trials per day from day 1 to day 5 and 4 training trials per day from day 6 to day 10.

Probe trials were conducted 5 hours after the final training trial on day 5 and day 10. In the absence of a hidden platform, the mice were released at the center of pool and were allowed to swim for 60 s. Both training and probe trials were conducted by the same operator. The latency to reach a hidden platform (escape latency) of each mouse was calculated as the mean from the six trials (day 1 to day 5) or the four trials (day 6 to day 10) within each day. The percentage of time staying in four quadrants (quadrant occupancy) was measured during probe trials.

Licking test and data recording

Thirsty mice showed vigorous activity when water was available, and they drank from different angles either in front of or under the water nozzle. This tendency reduced the accuracy of licking recording. Thus, an apparatus that monitors neural circuitries while

a mouse is licking (Komiyama et al., 2010) was utilized. The apparatus (TaskForcer; O'Hara & Co., Ltd, Tokyo, Japan) for licking training and data recording includes a water-pumping device and an infrared beam detector system, which are controlled by software (OPR-9210). A custom-made head plate was fixed onto a mouse's skull with dental acrylic to reduce its head movement. After 2 days of water deprivation, the mouse was placed inside an acrylic tube and trained to lick for a water reward for 15 min per day for 7 consecutive days. Each interruption of the infrared beam counted as one lick, and the mouse was rewarded with one unit of water (4 μl of water per lick). From the beginning of training, the water intake per day was restricted to 1.5 ml until the end of the experiment. If a mouse failed to acquire 1.5 ml water during the training or testing session (which was typical), the remaining amount of water was provided by a water dispenser after the session. For these experiments, a burst was defined as continuous licking (≥ 2 licks) with < 0.4 s between licks. Bursts were recorded only within the first minute after the first lick.

Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM) and were analyzed with GraphPad Prism (version 7; GraphPad Software, San Diego, CA, USA). The normality of all data was checked by a chi-square goodness-of-fit test ($p > 0.05$). The DA concentration of dialysates, optical density of TH, water consumption per day, and force by four limbs were compared between genotypes using one-way analyses of variance (ANOVAs). The effects of genotype or place on the quadrant occupancy were analyzed by two-way ANOVAs. For data across days, repeated-measures (RM)-ANOVAs, with time serving as a within-group factor and genotype serving as a between group factor, were performed to examine the effects of factors and the

interaction between the effects of two factors. The main effect of time was further assessed by a *post hoc* test (Dunnett's test), which compared the first day with each individual day. When the interaction was significant (genotype × time), a comparison of genotypes (Sidak test) at each individual day. A p value of <0.05 was considered statistically significant.

Results

DSI mice were mostly indistinguishable from control littermates but had lower DA concentrations in the striatum and NAc

A new transgenic mouse (DSI mouse) line with Cre-mediated cell type-specific expression of tetX light chain in DAergic neurons throughout the brain (Fig. 1.1A, B) was used in this study. In these mice, tetX light chain expression following tamoxifen-induced Cre-loxP recombination prevents neurotransmitter release from DAergic neuron axon terminals by cleaving v-SNAREs (Fig. 1.1C). DA concentrations in the striatum and NAc regions of these mice were suppressed to 61.4% ± 13.4% (mean ± SEM) [ANOVA: $F(1,10) = 5.576$, $p = 0.040$] and 54.5% ± 11.7% [ANOVA: $F(1,10) = 5.710$, $p = 0.038$], respectively (Fig. 1.2A–H), of that in control mice 6 weeks after tamoxifen administration. To examine if the expression of TH in DAergic neurons was altered in response to the DA loss, the optical density of anti-TH immunostaining signals in the VTA and the SN was calculated; however, there was no statistical significance between the control and DSI mice [ANOVA: VTA, $F(1,4) = 0.082$, $p = 0.79$; SN, $F(1,4) = 0.648$, $p = 0.47$] (Fig. 1.3A–C). The EYFP signal triggered by the Cre-loxP recombination overlapped the TH signal (Fig. 1.4A, B), confirming the cell specificity of transgene expression, with 51.8% ± 1.2% (mean ± SEM) of the TH^+ cells in the VTA and 46.2% ± 1.5% of the TH^+ cells in the SN expressing EYFP (Fig. 1.4C).

Figure 1.5A shows the body weights of control and DSI mice after tamoxifen administration (Ctrl, $n = 10$; DSI, $n = 10$). The RM-ANOVA comparing body weights across genotypes revealed no significant effect of genotype or interaction between genotype and time [body weight, $F(1,18) = 0.024$, $p = 0.88$; genotype × time, $F(3,54) = 0.533$, $p = 0.66$], whereas a significant effect of time was observed [$F(3,54) = 10.78$, p

< 0.001], indicating that no difference in weight gain existed between control and DSI mice. The DSI mice also consumed the same amount of water as control littermates (Fig. 1.5B) (Ctrl, n = 14; DSI, n = 14) (ANOVA: $F(1,26) = 1.213$, $p = 0.28$).

DSI mice held the ability to complete different tasks

During training (day 1 to day 3) on the rotarod (Fig. 1.6A), there was no significant effect of genotype on the latency to fall (Ctrl, n = 10; DSI, n = 10) (Fig. 1.6B) [RM-ANOVA: genotype, $F(1,18) = 0.011$, $p = 0.92$; time, $F(2,36) = 65.155$, $p < 0.001$; genotype × time, $F(2,36) = 0.143$, $p = 0.87$]. During testing sessions (day 4 to day 8), the DSI mice fell from the rotating rod earlier than the control mice [RM-ANOVA: genotype, $F(1,18) = 6.242$, $p = 0.02$; time, $F(4,72) = 24.730$, $p < 0.001$; genotype × time, $F(4,72) = 3.211$, $p = 0.018$], indicating that the motor control in DSI mice might have been impaired. However, the grip strength test revealed that the DSI mice had the same limb strength as control mice (Fig. 1.6C) [ANOVA: $F(1,18) = 0.107$, $p = 0.75$]. As the interaction between the effects of genotype and time on the latency to fall was significant and the significant differences between genotypes revealed by *post hoc* tests were only under challenging situations (rotating speed ≥ 28 rpm) (Sidak test, Ctrl vs. DSI: day 7 [28 rpm], $p = 0.004$; day 8 [32 rpm], $p = 0.021$), it was concluded that the DSI mice still had adequate body strength to perform different behaviors.

The mice were trained to remember and find the location of a hidden platform in a pool filled with opaque water (Fig. 1.7). During spatial learning session (day 1 to day 5), there was no significant effect of genotype on the escape latency (Ctrl, n = 7; DSI, n = 7) (Fig. 1.8A) [RM-ANOVA: genotype, $F(1,12) = 0.062$, $p = 0.81$; time, $F(4,48) = 26.133$, $p < 0.001$; genotype × time, $F(4,48) = 0.114$, $p = 0.98$]. The 5-day consecutive training significantly reduced the escape latency of mice (*post hoc*

Dunnett's test: day 1 vs. day 2, $p < 0.05$; day 1 vs. day 3, $p < 0.001$; day 1 vs. day 4, $p < 0.001$; day 1 vs. day 5, $p < 0.001$). The probe trail on day 5 revealed that both control and DSI mice tended to stay in the NE quadrant, where the hidden platform was placed during training session (day 1 to day 5) [two-way ANOVA: genotype, $F(1,48) < 0.001$, $p > 0.99$; place, $F(3,48) = 72.860$, $p < 0.001$; genotype × place, $F(3,48) = 1.058$, $p = 0.38$] (*post hoc* Dunnett's test: NE vs. NW, $p < 0.001$; NE vs. SE, $p < 0.001$; NE vs. SW, $p < 0.001$). Since there was no significant difference between control and DSI mice, it was suggested that the spatial learning of DSI mice are intact.

The hidden platform was relocated to the opposite quadrant (Fig. 1.7) during reversal learning session (day 6 to day 10). The RM-ANOVA revealed no significant effect of genotype on the escape latency (Ctrl, $n = 7$; DSI, $n = 7$) (Fig. 1.8B) [RM-ANOVA: genotype, $F(1,12) = 0.008$, $p = 0.93$; time, $F(4,48) = 19.365$, $p < 0.001$; genotype × time, $F(4,48) = 0.438$, $p = 0.78$]. The escape latency of mice significantly decreased after 5-day consecutive training (*post hoc* Dunnett's test: day 6 vs. day 8, $p < 0.001$; day 6 vs. day 9, $p < 0.001$; day 6 vs. day 10, $p < 0.001$). The probe trail on day 10 indicated that both control and DSI mice tended to stay in the SW quadrant, where the hidden platform was relocated during training session (day 6 to day 10) [two-way ANOVA: genotype, $F(1,48) < 0.001$, $p > 0.99$; place, $F(3,48) = 23.640$, $p < 0.001$; genotype × place, $F(3,48) = 0.150$, $p = 0.93$] (*post hoc* Dunnett's test: SW vs. NE, $p < 0.001$; SW vs. NW, $p < 0.001$; SW vs. SE, $p < 0.001$). This result suggested that DSI mice were able to learn the new location of platform as control mice do.

DSI mice lick less for a water reward under water-deprived conditions

The influence of moderate DA loss on drinking behavior was investigated under conditions of water deprivation, which provides the motivation to drink. After 2 days of

water deprivation (Fig. 1.9A), the mice were placed into a lick-measuring device with a water-pumping pipe for behavioral training (Ctrl, n = 16; DSI, n = 16). The RM-ANOVA revealed main effects of genotype and time (Fig. 1.9B) [genotype, $F(1,30) = 6.506$, $p = 0.016$; time, $F(6,180) = 55.226$, $p < 0.001$], but the interaction between these factors was not statistically significant [genotype × time, $F(6,180) = 1.898$, $p = 0.083$]. The results indicated that 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 and that the DSI mice licked significantly less than the control mice.

Figures

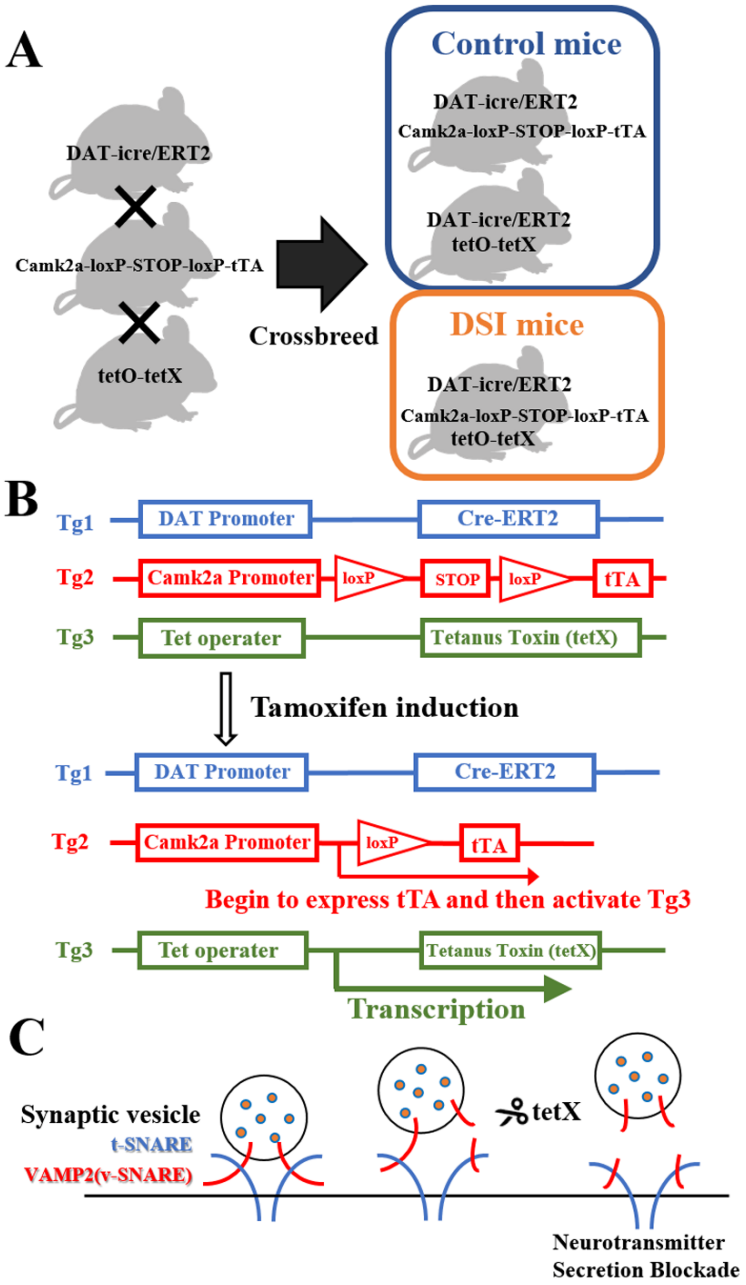

Figure 1.1 Transgenic construction of the DSI mice

(A) Breeding scheme to generate DSI mice. **(B)** In Tg1, Cre recombinase is expressed under the control of the promoter for the *Slc6a3* (DAT) gene, which is specifically active in DAergic neurons. In Tg2, tTA protein expression is regulated by the α-CaMKII promoter and Cre-loxP recombination. In Tg3, tetX light chain, which interferes with neurotransmitter release, is expressed under the control of the tetracycline (Tet) operator. Tamoxifen administration induces Cre-loxP recombination in DAergic neurons and then the expression of tTA, resulting in the activation of Tg3. **(C)** The tetX cleaves v-SNARE, thereby preventing synaptic vesicles that store neurotransmitters from releasing their contents into the synapse.

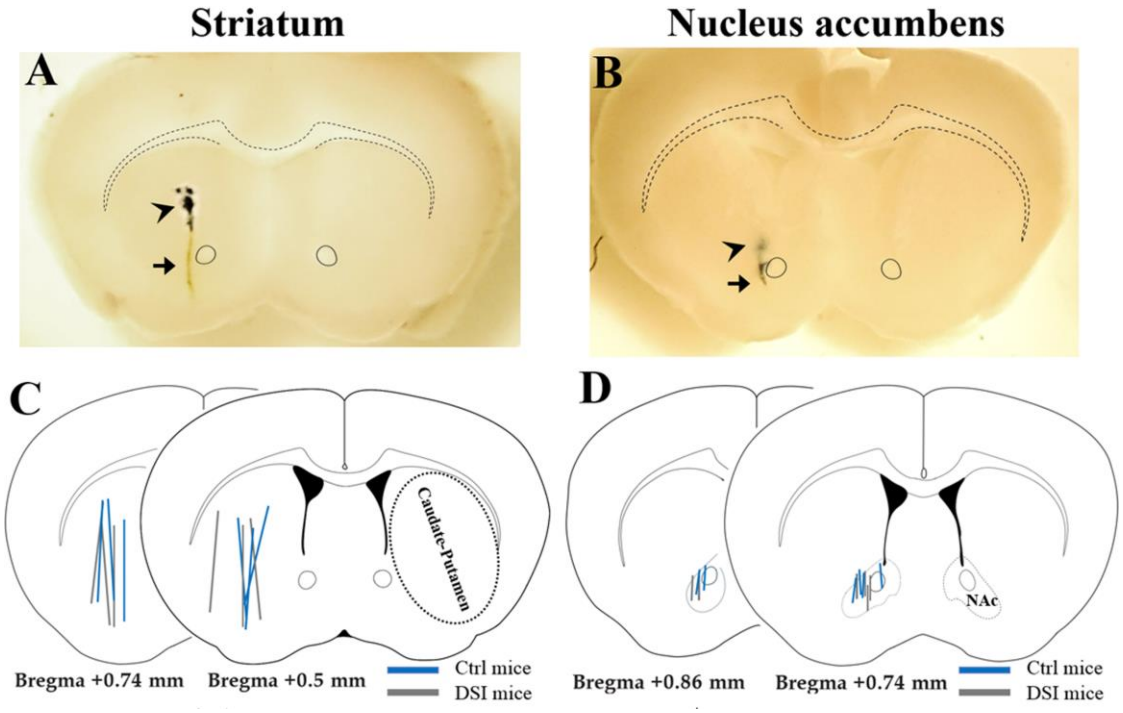

Figure 1.2(A-D) Microdialysis and DA concentrations in DSI and control mice
Representative coronal sections and schematic drawings showing the site of the implanted microdialysis probe for measuring the DA concentration in the striatum **(A, C)** or in the NAc **(B, D)**. The arrowheads indicate the ends of the guide cannulae marked by Evans blue, and the arrows point to the traces left by the microdialysis probes.

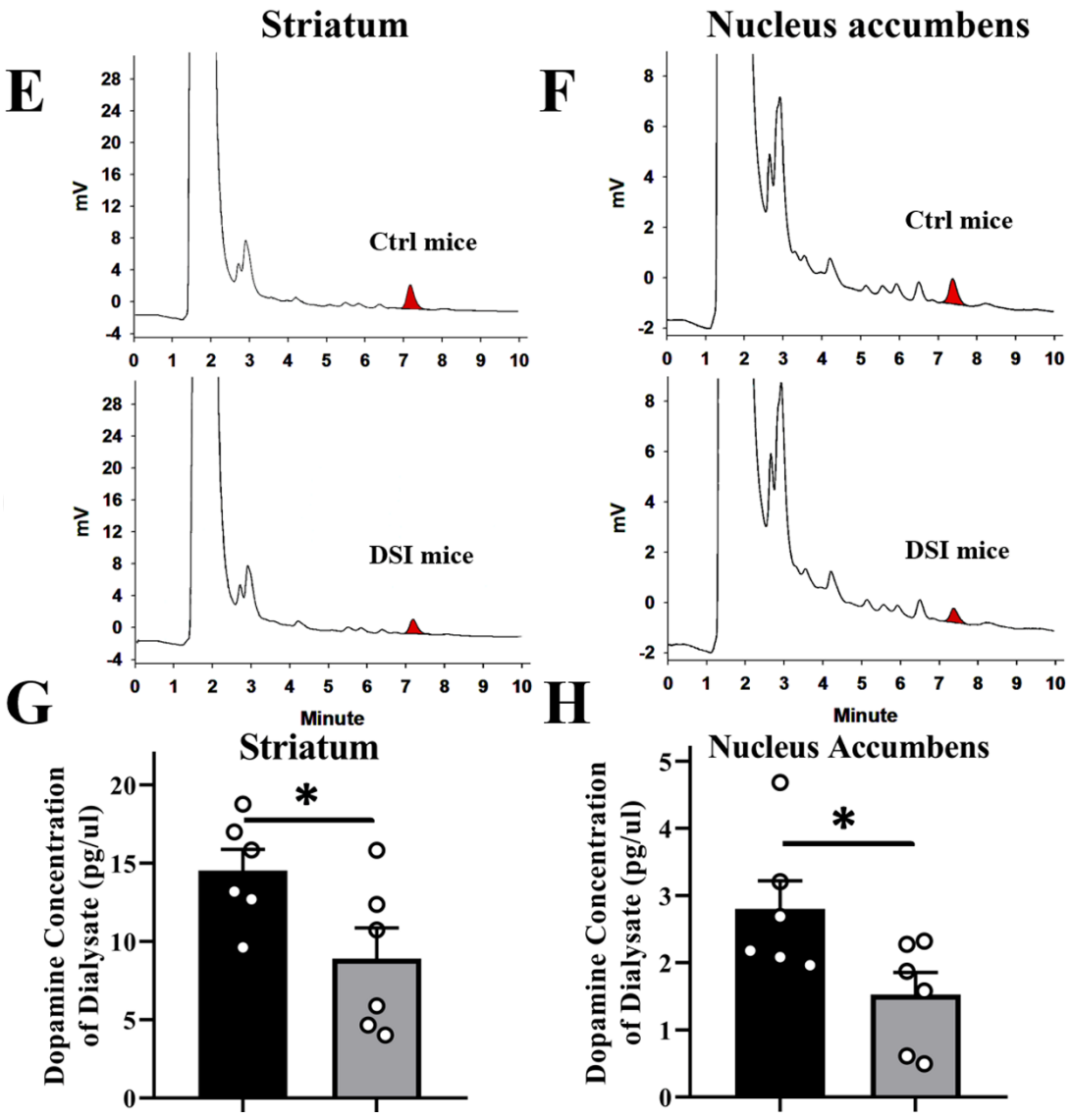

Figure 1.2(E-H) Microdialysis and DA concentrations in DSI and control mice
Six to eight weeks after tamoxifen administration, the DA concentrations of the dialysates collected from the striatum **(E, G)** (61.4% ± 13.4% [mean ± *SEM*]; ANOVA, $p < 0.05$) or the NAc **(F, H)** (54.5% ± 11.7%; ANOVA, $p < 0.05$) were compared between the control and DSI mice. (both, Ctrl, $n = 6$; DSI, $n = 6$) **(E, F)** The peaks of DA in representative chromatograms are in red. *$p < 0.05$. Values are shown as the means ± SEMs.

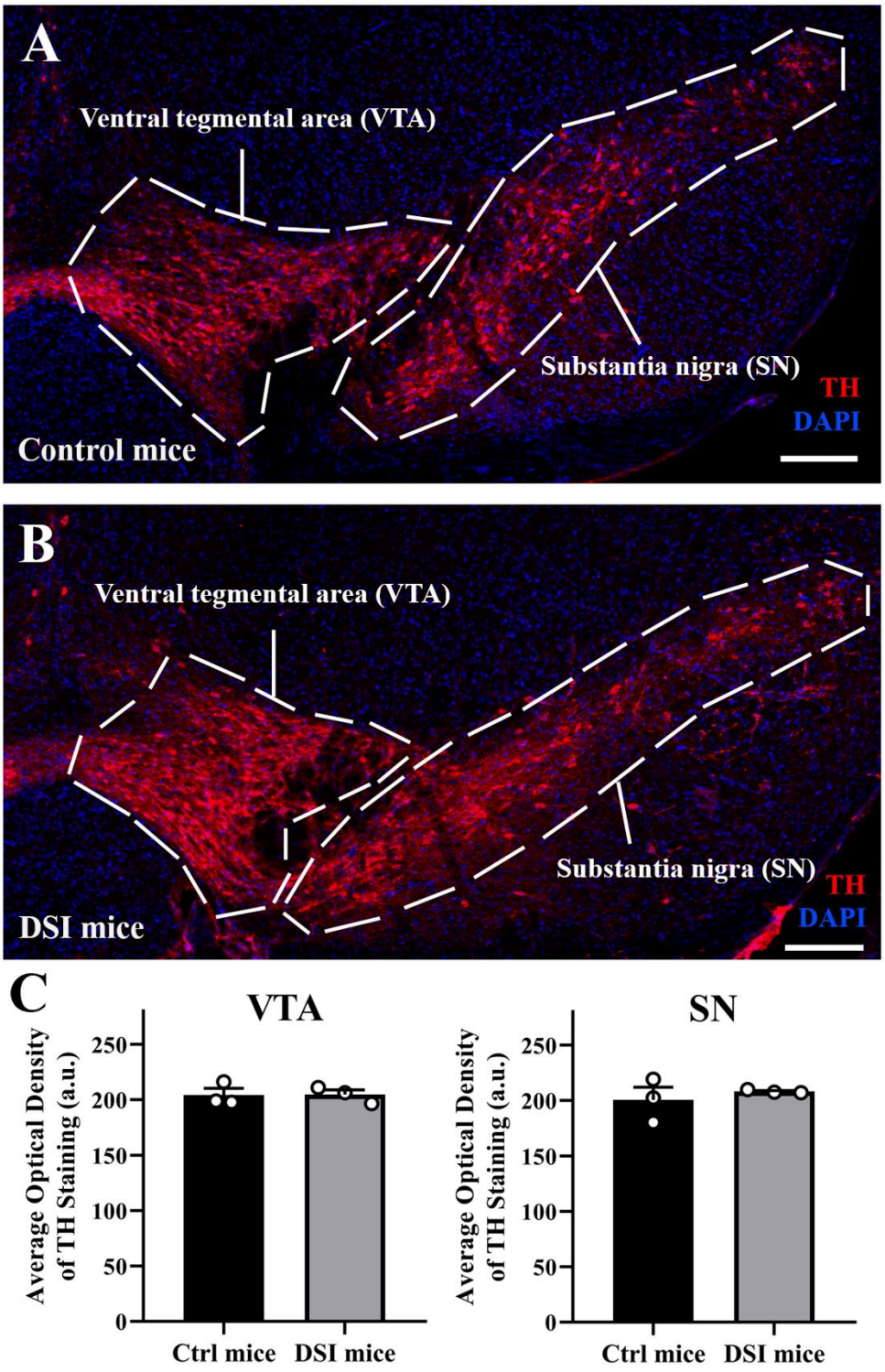

Figure 1.3 Immunohistochemistry of DSI and control mice
Representative image of the TH staining of a control **(A)** or DSI **(B)** mouse near Bregma -3.6 mm. Scale bars, 150 μm. Most of the DAergic neurons were inside the VTA or the SN. **(C)** The optical densities of TH signals in VTA or SN were calculated and compared between the DSI and control mice (ANOVA: VTA, $p = 0.79$; SN, $p = 0.47$) (Ctrl, $n = 3$; DSI, $n = 3$). Values are shown as the means ± SEMs.

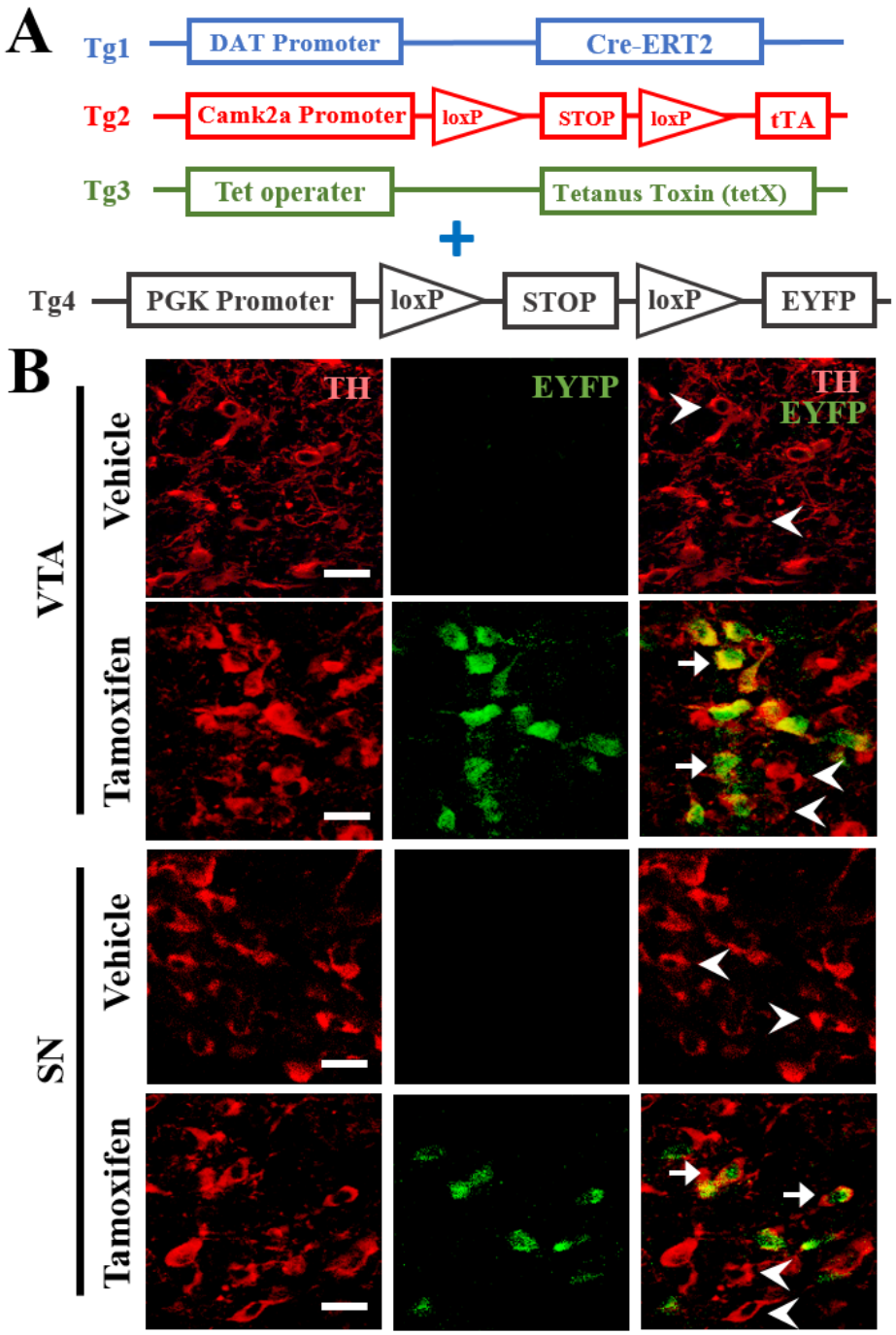

Figure 1.4(A-B) Cre-loxP recombination in the DSI mice

(A) A fourth transgene (Tg4) was introduced into the DSI mouse to locate the cells that underwent Cre-loxP recombination. **(B)** Representative images of TH (red) and EYFP (green) double staining in the VTA or SN of a DSI mouse carrying the Tg4 transgene were taken 6 weeks after tamoxifen or vehicle administration. The arrowheads indicate the TH^+ cells, and the arrows point to the $TH^+/EYFP^+$ cells. Scale bars, 25 μm. $EYFP^+$ cells overlapped with the DAergic neurons (TH^+) in the quadruple transgenic mice. (DSI: vehicle, $n = 4$; tamoxifen, $n = 4$)

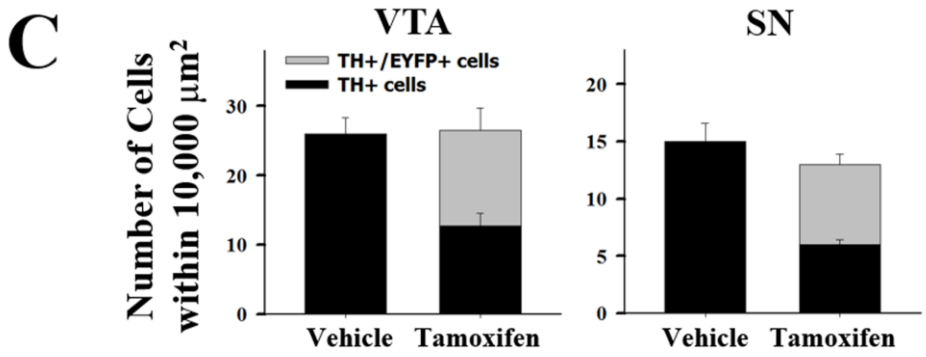

Figure 1.4(C) Cre-loxP recombination in the DSI mice
Within the VTA or SN, the average numbers of the $TH^+/EYFP^+$ cells and the TH^+ cells in the areas of 10,000 μm^2 were calculated. (DSI: vehicle, $n = 4$; tamoxifen, $n = 4$) Values are shown as the means ± SEMs.

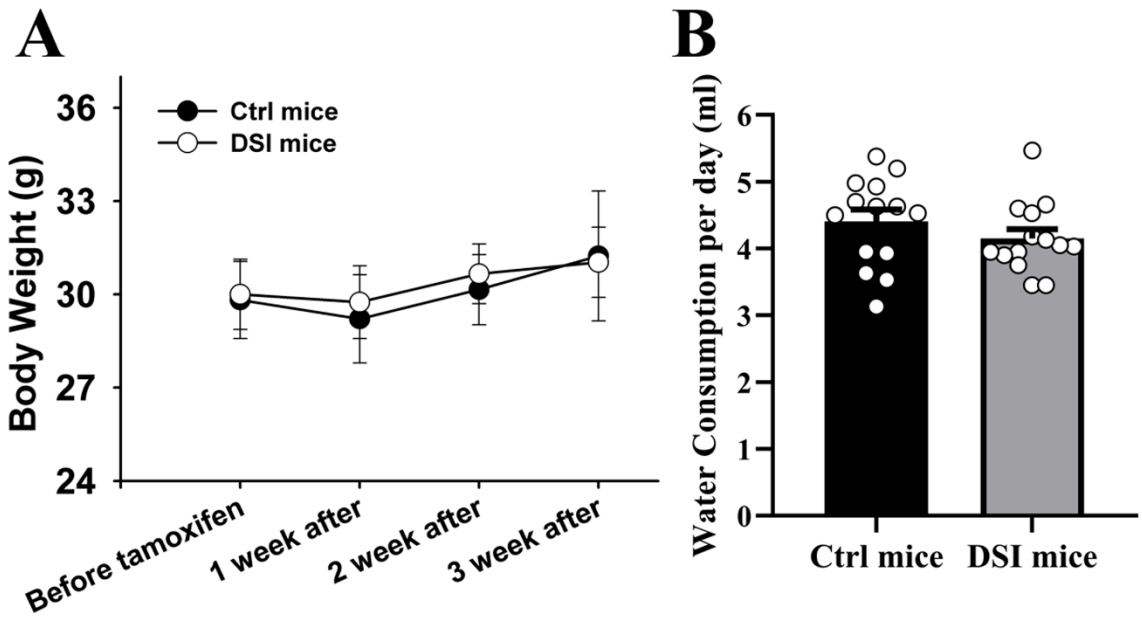

Figure 1.5 Body weights and water consumption of DSI and control mice
Mice were provided with food and water *ad libitum*. **(A)** Body weight was recorded from the first day of tamoxifen administration to 3 weeks later (RM-ANOVA: genotype, p = 0.88) (Ctrl, n = 10; DSI, n = 10). **(B)** Water intake was recorded starting the second week after tamoxifen treatment, for two weeks (ANOVA, p = 0.28) (Ctrl, n = 14; DSI, n = 14). Values are shown as the means ± SEMs.

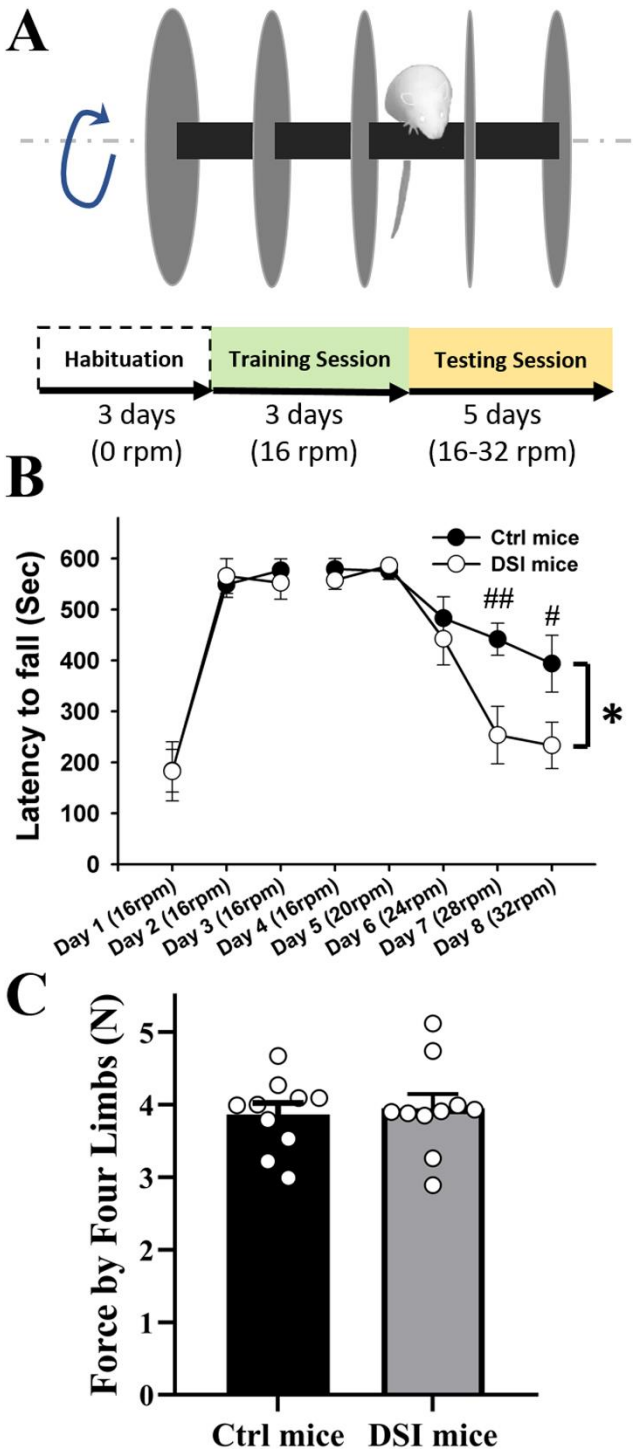

Figure 1.6 Motor control of DSI and control mice

(A) Scheme of the rotarod test. **(B)** After 3 days of training (day 1 to day 3) (RM-ANOVA: time, $p < 0.01$), control and DSI mice were challenged with various rotation speeds (16–32 rpm) on subsequent days (day 4 to day 8) (RM-ANOVA: genotype, $p < 0.05$; time, $p < 0.01$; genotype × time, $p < 0.05$). **(C)** Comparison of the average four-limb grip strength between control and DSI mice (ANOVA, $p = 0.75$) (Ctrl, $n = 10$; DSI, $n = 10$). *$p < 0.05$ compared to Ctrl mice. #$p < 0.05$ and ##$p < 0.01$ compared within a day. Values and are shown as the means ± SEMs.

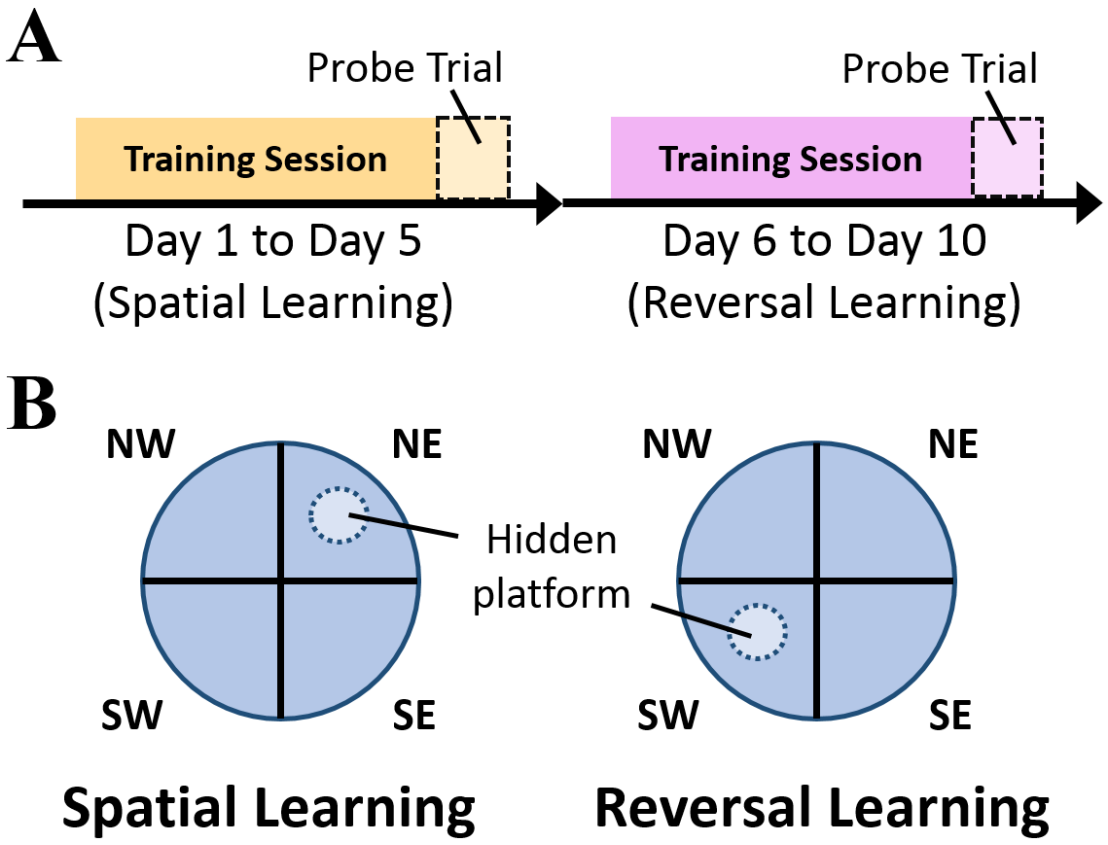

Figure 1.7 Scheme of the Morris water maze
(A) Protocol for spatial learning (day 1 to day 5) and reversal learning (day 6 to day 10). Probe trials were conducted 5 hours after the final training trial on day 5 and day 10. **(B)** Control and DSI mice were trained to find a hidden platform 1 cm below the water surface. The platform was placed in the NE quadrant from day 1 to day 5 and relocated to the SW quadrant from day 6 to day 10. The probe trials were conducted in the absence of the platform.

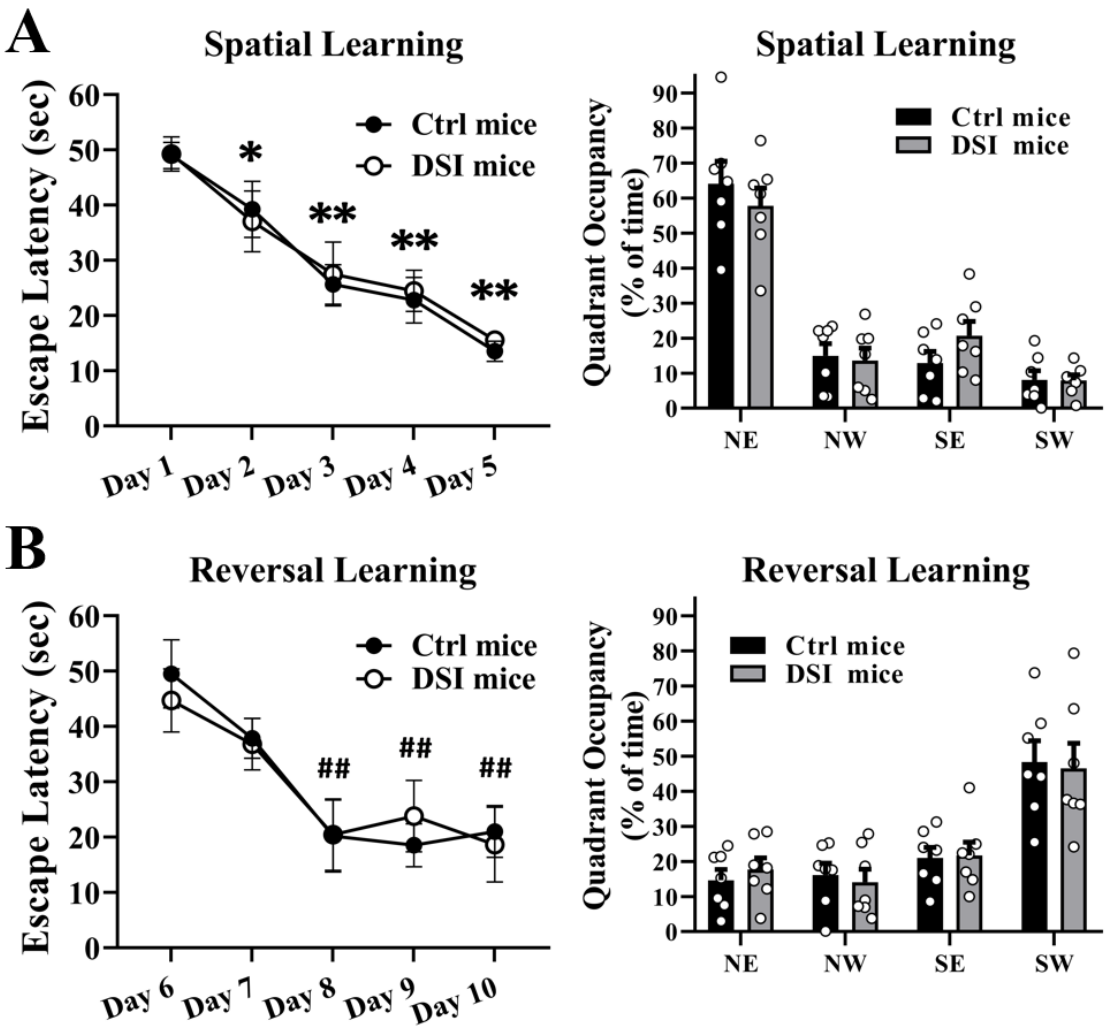

Figure 1.8 Spatial and reversal learning of DSI and control mice

Control and DSI mice were released from four pseudorandomly assigned start locations (NW, NE, SW, and SE) and allowed to swim for 60 s. The performance of spatial learning **(A)** and reversal learning **(B)** of mice were evaluated by the escape latencies to reach the hidden platform during training sessions (left) and the quadrant occupancy during probe trails (right). (Ctrl, n =7; DSI, n = 7). (RM-ANOVA: genotype, $p > 0.81$; time, $p < 0.01$). *$p < 0.05$ and **$p < 0.01$ compared to day 1. ##$p < 0.01$ compared to day 6. Values are shown as the means ± SEMs.

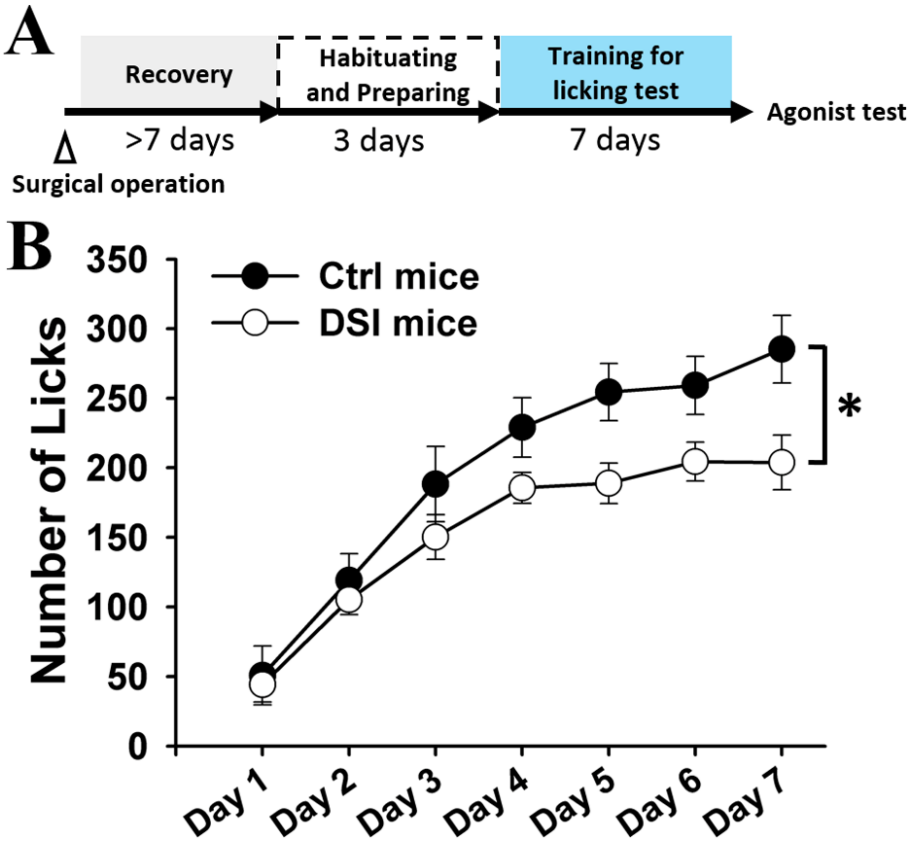

Figure 1.9 Training mice to lick for a water reward

(A) Scheme of the training for licking test. **(B)** After 2 days of water deprivation, control and DSI mice were trained to lick a water nozzle for a water reward (4 μl/lick) (RM-ANOVA: genotype, $p < 0.05$; time, $p < 0.01$). The daily water intake was limited to 1.5 ml per day, and the body weight was maintained at the same level (Ctrl, n =16; DSI, n = 16). *$p < 0.05$ compared to Ctrl mice. Values are shown as the means ± SEMs.

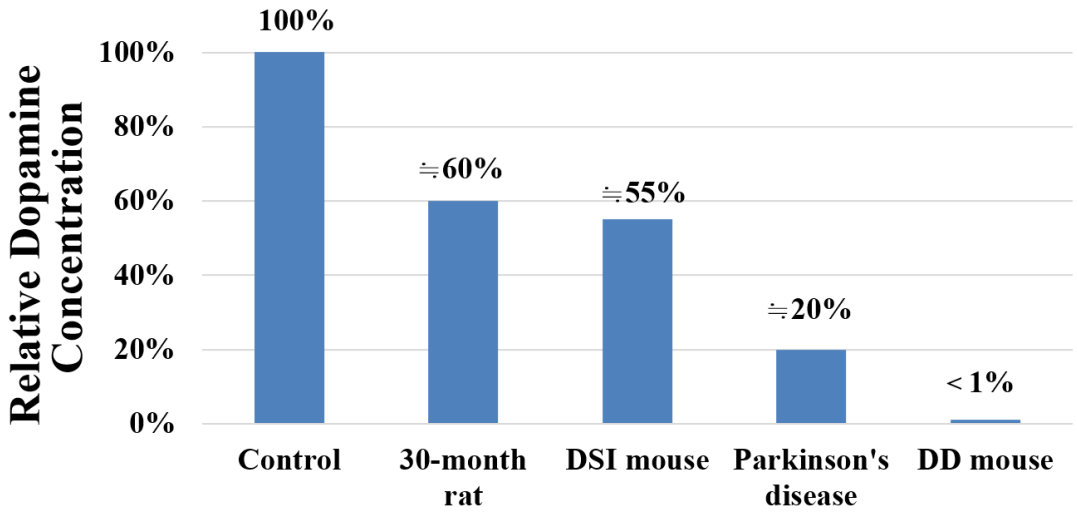

Figure 1.10 Reduction of DA concentration by different causes
DA concentration in central nerve system is reported to decrease while animals grow old (Friedemann & Gerhardt, 1992), and progression of some diseases, such as Parkinson's disease (Zarow, Lyness, Mortimer & Chui, 2003), also accompanies reduction of DA concentration. Although DSI mouse line may not be a suitable animal model for the study of aging or Parkinson's disease, findings by DSI mice may help understand the causes of some features.

Discussion

DSI mice had lower DA concentrations in the brain after tamoxifen administration

Transgenic mouse completely lacking DA (i.e., DA-deficient mouse) is a valuable animal model for studying the role of DA in eliciting actions (Zhou & Palmiter, 1995; Palmiter, 2008) and has contributed to the knowledge of motivation. However, such mice consume less food and water than their control littermates, making it difficult to evaluate the influence of DA on certain behaviors. Therefore, a more suitable triple transgenic mouse model (i.e., the DSI mice) with suppressed DAergic signaling was generated.

DSI mice harbor the DAT-icre/ERT2 transgene (Tg1), which enables expression of tamoxifen-inducible Cre recombinase in DAergic neurons via the *Slc6a3* promoter (Gore et al., 2017; Schriever et al., 2017). Tamoxifen administration resulted in Cre-loxP recombination in approximately one-half of DAergic (TH^+) neurons (Fig. 1.4G, H). The expression of tetX light chain (Tg3) to block synaptic release is indirectly regulated by an α-CaMKII promoter (Tg2), whose activity is weak in DAergic neurons (Burgin *et al.*, 1990; Wang *et al.*, 2013). Thus, some DAergic neurons whose loxP-STOP-loxP cassette (Tg2) has been removed may not produce sufficient amounts of tetracycline transactivator, resulting in incomplete blockade of DA secretion. As a result, the DA concentrations in mice received tamoxifen administration were reduced by 38.6% in the striatum and by 45.5% in the NAc (Fig. 1.2C), and the ratio of DA concentration reduction was slightly smaller than the ratio of DAergic neurons which underwent the Cre-loxP recombination.

Neurotransmitter secretion interference was restricted to the DAergic neurons in

DSI mice. This may apply to DA and gamma-aminobutyric acid (GABA), which is also produced by some TH^+ cells (Borisovska, Bensen, Chong, & Westbrook, 2013; Tritsch, Ding, & Sabatini, 2012). However, dual-transmitter neurons are relatively rare, and some are considered nonexocytotic because they lack vesicular monoamine transporter (Peter et al., 1995; Weihe, Depboylu, Schütz, Schäfer, & Eiden, 2006). Hence, the results observed in DSI mice were attributed to the reduced DA secretion.

DSI mice had similar body weights and water consumption as littermate controls and exhibited motor control impairment only under a challenging situation. These findings demonstrate the usefulness of DSI mice to study the role of DA in diverse behaviors. The result of rotarod test indicate that DAergic inputs in the striatum of DSI mice are sufficient to relieve the inhibitory effect of GABAergic neurons on movement (Gerfen, 1992) but inadequate to attune striatal output neurons during intense activity. As the DA-deficient mice were shown to be able to learn the location of food without DA (Robinson et al., 2005), DSI mice had no difficulty in learning and finding the location of a hidden platform in Morris water maze. It is suggested that DSI mice may hold equivalent or even better learning capacity than DA-deficient mice because DSI mice still have functioning DA systems. However, it is unclear if the DSI mice used the same strategy as do the control mice to find the hidden platform. The findings by DSI mice may also contribute to the study of behavioral change related to aging (Fig. 1.10) (Flood & Coleman, 1988; Friedemann & Gerhardt, 1992), since the DA concentration in the striatum is reported to decrease while aging.

Licking microstructure analysis revealed altered water drinking behavior in DSI mice

The numbers of total licks indicate the degree of feeding activity (Fig. 1.9A) and thus is

commonly used to evaluate changes in fluid ingestion and general drinking behavior (D'Aquila, Rossi, Rizzi, & Galistu, 2012; Davis, 1989; Higgs & Cooper, 1998; Mendez et al., 2016). Moderate loss of DA resulted in fewer licks in water-deprived DSI mice (Fig. 1.9B), indicating a suppression of water drinking. As the number of licks is representative of general drinking behavior, the fewer licks by DSI mice suggest changes to the drinking behavior. Importantly, there was no significant difference between control and DSI mice in long-term *ad libitum* water consumption (Fig. 1.5B), indicating that the homeostatic control in DSI mice remained intact.

The DA-deficient mice manifest the preference for sweet solution in the absence of DA as do control mice but less frequently initiate licking (Cannon & Palmiter, 2003); however, the DA-deficient mice lick faster and emit more licks than the control mice once the licking is initiated. The transient, intense licking of DA-deficient mice may reflect the hypersensitivity in MSNs or the adaptations in other neurons to the lack of DA. By contrast, the DSI mice are also able to learn the association between water nuzzle and reward, and the performance of DSI mice nearly matches that of control littermates. The DSI mice should be spared from the hypersensitivity shown by the DA-deficient mice because frequent injection of L-DOPA (providing continuous DAergic stimulation) reversed the hypersensitivity in DA-deficient mice (Kim et al., 2006). Nevertheless, it remains unclear if the hedonic impact brought by the taste of reward that reinforces behavior is affected by DA loss in either DA-deficient mice or DSI mice. Further tests providing different concentrations of rewards are required to examine this possibility, e.g. sucrose, saccharine, and salt solution.

Chapter 2

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

Introduction

The analysis of licking microstructure has been proved to be a very useful method to study behavioral change from the perspectives of 'liking' (hedonic impact of reward) and 'wanting' (incentive motivation) (Berridge, 1996; Taha & Fields, 2005; Berridge *et al.*, 2009; Uematsu *et al.*, 2011; Davies *et al.*, 2015; D'Aquila & Galistu, 2017; Dastugue *et al.*, 2018; Johnson, 2018). The meanings of licking (Fig. 2.1) are based on the observation made by J. Davis (Davis & Smith, 1992) and the incentive salience attribution hypothesis (Berridge, 2018; D'Aquila & Galistu, 2017; Johnson, 2018). The numbers of total licks indicate the degree of feeding activity and thus is commonly used to evaluate changes in fluid ingestion and general drinking behavior (Davis, 1989; Higgs & Cooper, 1998; D'Aquila *et al.*, 2012; Mendez *et al.*, 2016). Rodents usually cluster their licks into separate sets known as bursts or bouts. The size of bursts represents the hedonic impact brought by the taste and reflects the orosensory positive feedback (Dwyer, 2012; Kosheleff et al., 2018; Mendez, Ostlund, Maidment, & Murphy, 2015; Ostlund, Kosheleff, Maidment, & Murphy, 2013), and the intra-burst lick speed is used as an indicator of licking-associated motor control to reveal the effect on tongue movement (Gramling *et al.*, 1984; Gramling & Fowler, 1986). The number of bursts reflects the incentive motivation triggered by cues because it indicates the activation of responses and is highly affected by orosensory and post-ingestive mechanisms (Davis & Smith, 1992; Johnson et al., 2010, 2013; Smith, 2001).

Injection of DA into brain regions regulating thirst was reported to reduce the water intake in rats (Miyahara et al., 2012; Tonelli & Chiaraviglio, 1995). It has been indicated that DA receptors modulate drinking behavior, but that D1-like and D2-like receptors may differentially influence the incentive motivation in rodent or the hedonic

impact of reward. P. S. D'aquila and A. Galistu suggested that DA D1-like receptors play a role in the activation of reward-associated responses (D'Aquila, 2010; D'Aquila *et al.*, 2012; Galistu & D'Aquila, 2012, 2013) and DA D2-like receptors are involved in the evaluation of reward (Schneider *et al.*, 1990; Canu *et al.*, 2010; D'Aquila, 2010; D'Aquila *et al.*, 2012; Galistu & D'Aquila, 2013). Their hypothesis regarding the role of DA receptors in fluid ingestion was based on the effects of DA receptor antagonists on rats' licking microstructure (D'Aquila & Galistu, 2017; Johnson, 2018a). Thus far there is only one study that assessed the effect of DA receptor agonist on licking microstructure (Genn et al., 2003), and it showed an inhibitory effect of D2/D3 receptor agonist on the licking for sucrose solution. However, the effect of agonist on mice with moderate DA loss has not been examined.

In this chapter, I investigated the role of DA receptors in water drinking behavior by analyzing licking microstructures (number of licks and bursts, size of bursts and intra-burst lick speed). To compensate for the moderate DA loss in the DSI mice, I administered a DA D1 receptor agonist (SKF38393 or A68930) or a DA D2/D3 receptor agonist (ropinirole) subcutaneously before measuring the water drinking behavior.

Materials and Methods

Animals and drug treatment

The DSI mouse harbors *Slc6a3*(DAT)-icre/ERT2 (Tg1), *Camk2a*-loxP-STOP-loxP-tTA (Tg2), and tetO-tetX (Tg3) constructs. The DSI mice without *Camk2a*-loxP-STOP-loxP-tTA or tetO-tetX were used as control (Ctrl) mice. Three to four mice per cage maintained under a 12 h:12 h light-dark cycle (lights on at 08:00 a.m.) at 22°C were given *ad libitum* access to food and water. All animal procedures and experiments in this study were approved by the Ethical Committee of the University of Tokyo and were conducted according to the Guidelines for Animal Experimentation required by the University of Tokyo.

Tamoxifen (350 mg/kg, dissolved in corn oil) was orally administered to control and DSI mice (male, littermates, 12 weeks old) for 4 consecutive days, and the last administration was given at least 1 month before the behavioral experiments. Mice received subcutaneous injections of DA D1 receptor agonist A68930 [*cis*-(±)-A68930 hydrochloride; Tocris Bioscience] or SKF38393 [(±)-SKF38393 hydrochloride; Sigma-Aldrich] or D2/D3 receptor agonist ropinirole (ropinirole hydrochloride; FUJIFILM Wako Pure Chemical Corp.) dissolved in saline. These injections of A68930 (0.01, 0.24, and 1.2 mg/kg), SKF38393 (0.24, 6, and 30 mg/kg), ropinirole (0.2, 4.8, and 10 mg/kg), or saline (vehicle) were administered 5 min before the behavioral experiments.

Licking test and data recording

The apparatus (TaskForcer; O'Hara & Co., Ltd, Tokyo, Japan) for licking training and data recording includes a water-pumping device and an infrared beam detector system,

which are controlled by software (OPR-9210). A custom-made head plate was fixed onto a mouse's skull with dental acrylic to reduce its head movement. After 2 days of water deprivation, the mouse was placed inside an acrylic tube and trained to lick for a water reward for 15 min per day for 7 consecutive days. Each interruption of the infrared beam counted as one lick, and the mouse was rewarded with one unit of water (4 μl of water per lick). From the beginning of training, the water intake per day was restricted to 1.5 ml until the end of the experiment. If a mouse failed to acquire 1.5 ml water during the training or testing session (which was typical), the remaining amount of water was provided by a water dispenser after the session. For these experiments, a burst was defined as continuous licking (≥ 2 licks) with < 0.4 s between licks. Bursts were recorded only within the first minute after the first lick.

The day after the final training session, mice received a subcutaneous injection of saline (vehicle) 5 min prior to the testing session, for which the setting was the same as the training session, but the time was reduced to 10 min. On subsequent days, the mice were assigned to receive A68930, SKF38393, or ropinirole at escalating concentrations every 2 days across sessions (A68930: 0.01, 0.24, and 1.2 mg/kg; SKF38393: 0.24, 6, and 30 mg/kg; ropinirole: 0.2, 4.8, and 10 mg/kg). The day after each drug injection, the mice were injected with saline to prevent the carryover of drug effects.

Statistical analysis

All data are expressed as the mean ± SEM and were analyzed with GraphPad Prism (version 7; GraphPad Software, San Diego, CA, USA). The normality of all data was checked by a chi-square goodness-of-fit test ($p > 0.05$). For data across days, RM-ANOVAs, with agonist dose serving as a within-group factor and genotype serving as a between group factor, were performed to examine the effects of factors and the

interaction between the effects of two factors. The main effect of agonist dose (SKF38393, A68930, or ropinirole) was further assessed by a *post hoc* test (Dunnett's test), which compared the saline treatment group with each agonist dose group. When the interaction was significant (genotype × dose), a comparison of genotypes (Sidak test) at each agonist dose and a comparison of agonist dose (Dunnett's test) within each genotype were conducted by *post hoc* tests. A p value of <0.05 was considered statistically significant.

Results

DA D1 agonist treatment restored the number of licks by DSI mice

As in the training trials (Fig. 1.9B), a main effect of genotype was revealed by the RM-ANOVA comparing the numbers of total licks by mice treated with A68930 (Fig. 2.2A) (Ctrl, $n = 9$; DSI, $n = 9$) [genotype, $F(1,16) = 7.214$, $p = 0.016$], indicating that DSI mice still made fewer licks. Although the effect of A68930 treatment did not reach statistical significance [RM-ANOVA: dose, $F(3,48) = 2.288$, $p = 0.091$], a significant interaction was discovered between the effects of genotype and A68930 treatment [RM-ANOVA: genotype × dose, $F(3,48) = 3.972$, $p = 0.013$], indicating a differential effect of A68930 on control or DSI mice. To assess the origin of this interaction, the lick numbers were further compared within genotype or individual dose in *post hoc* tests. The number of licks by DSI mice increased with 1.2 mg/kg A68930 (highest dose) (*post hoc* Dunnett's test, 0 mg/kg vs. 1.2 mg/kg: Ctrl, $p = 0.78$; DSI, $p = 0.003$), and the increased lick number was close to that of control mice (*post hoc* Sidak test, Ctrl vs. DSI: 0 mg/kg, $p = 0.02$; 0.01 mg/kg, $p = 0.02$; 0.24 mg/kg, $p = 0.15$; 1.2 mg/kg, $p = 0.97$). The result suggests that the high dose of A68930 only affected the number of licks by DSI mice.

To avoid the influence of consumed water (post-ingestive feedback), only the bursts within the first minute after the first lick were recorded. The RM-ANOVA of the licking microstructure showed that the lick speeds within bursts and the sizes of bursts were not affected by genotype [intra-burst lick speed, $F(1,16) = 0.687$, $p = 0.42$; burst size, $F(1,16) = 0.002$, $p = 0.97$] or A68930 treatment [intra-burst lick speed, $F(3,48) = 0.930$, $p = 0.43$; burst size, $F(3,48) = 2.000$, $p = 0.13$] (Fig. 2.2B and C, respectively).

The RM-ANOVA revealed that the number of bursts was lower in the DSI mice

(Fig. 2.2D) [genotype, $F(1,16) = 5.399$, $p = 0.034$], and it was not affected by A68930 treatment [dose, $F(3,48) = 1.083$, $p = 0.37$; genotype × dose, $F(3,48) = 0.654$, $p = 0.58$].

Similarly to that for the A68930 treatment test, the RM-ANOVA comparing the numbers of total licks by mice treated with SKF38393 showed a main effect of genotype, indicating that DSI mice made fewer licks than control mice (Fig. 2.3A) (Ctrl, $n = 10$; DSI, $n = 10$) [genotype, $F(1,18) = 8.294$, $p = 0.010$]. SKF38393 treatment had a significant effect on the lick number [RM-ANOVA: dose, $F(3,54) = 5.857$, $p = 0.002$], but the interaction between the effects of two factors (genotype and agonist dose) did not reach statistical significance [RM-ANOVA: genotype × dose, $F(3,54) = 2.190$, $p = 0.100$]. The 30 mg/kg SKF38393 (highest dose) increased the numbers of licks regardless of genotype (*post hoc* Dunnett's test: 0 mg/kg vs. 30 mg/kg, $p = 0.001$).

Again, the lick speeds within bursts and the sizes of bursts remained unaffected by genotype [RM-ANOVA: intra-burst lick speed, $F(1,18) = 1.163$, $p = 0.30$; burst size, $F(1,18) = 0.627$, $p = 0.44$] or SKF38393 treatment [RM-ANOVA: intra-burst lick speed, $F(3,54) = 0.904$, $p = 0.45$; burst size, $F(3,54) = 2.134$, $p = 0.11$] (Fig. 2.3B and C, respectively).

The DSI mice had a decreased number of bursts (Fig. 2.3D) [RM-ANOVA: genotype, $F(1,18) = 9.086$, $p = 0.008$], but there was no significant effect of SKF38393 treatment or interaction between the effects of the two factors [RM-ANOVA: dose, $F(3,54) = 0.866$, $p = 0.46$; genotype × dose, $F(3,54) = 0.416$, $p = 0.74$].

DA D2/3 agonist ropinirole suppressed water drinking behavior in both control and DSI mice

RM-ANOVA comparing the numbers of total licks by mice treated with ropinirole revealed significant effects of genotype and ropinirole treatment on the lick number

(Fig. 2.4A) (Ctrl, $n = 7$; DSI, $n = 7$) [genotype, $F(1,12) = 5.893$, $p = 0.032$; dose, $F(3,36) = 39.588$, $p < 0.001$]. The interaction between the effects of the two factors did not reach statistical significance [RM-ANOVA: genotype × dose, $F(3,36) = 2.771$, $p = 0.06$]; however, treatment with ropinirole decreased the total number of licks regardless of genotype (*post hoc* Dunnett's test: 0 mg/kg vs. 4.8 mg/kg, $p < 0.001$; 0 mg/kg vs. 10 mg/kg, $p < 0.001$).

Although the effect of genotype on the lick speed within bursts and the size of bursts was not significant [RM-ANOVA, genotype: intra-burst lick speed, $F(1,12) = 0.107$, $p = 0.75$; burst size, $F(1,12) = 1.955$, $p = 0.19$], a main effect of ropinirole was observed [RM-ANOVA, dose: intra-burst lick speed, $F(3,36) = 7.502$, $p < 0.001$; burst size, $F(3,36) = 8.636$, $p < 0.001$] (Fig. 2.4B and C, respectively). The RM-ANOVA did not reveal a significant interaction between the effects of genotype and ropinirole treatment [intra-burst lick speed, $F(3,36) = 0.504$, $p = 0.68$; burst size, $F(3,36) = 0.787$, $p = 0.51$] (Fig. 2.4B and C, respectively); however, the *post hoc* test indicated that ropinirole treatment reduced the lick speed within bursts (Dunnett's test: 0 mg/kg vs. 10 mg/kg, $p = 0.001$) and the size of bursts (Dunnett's test: 0 mg/kg vs. 4.8 mg/kg, $p = 0.006$; 0 mg/kg vs. 10 mg/kg, $p = 0.011$) regardless of genotype.

The RM-ANOVA of the number of bursts revealed a main effect of ropinirole treatment (Fig. 2.4D) [dose, $F(3,36) = 48.143$, $p < 0.001$], while the effect of genotype and the interaction between the effects of the two factors did not reach statistical significance [genotype, $F(1,12) = 0.411$, $p = 0.53$; genotype × dose, $F(3,36) = 0.660$, $p = 0.58$]. The results indicate that the numbers of bursts in both the control and DSI mice were suppressed by ropinirole treatment at the two highest doses (*post hoc* Dunnett's test: 0 mg/kg vs. 4.8 mg/kg, $p < 0.001$; 0 mg/kg vs. 10 mg/kg, $p < 0.001$).

Table and Figures

	Lick Number	Intra-burst Lick speed	Burst Size	Burst Number
DSI mice	↓	→	→	↓
DSI mice + D1 agonist	→†	→	→	↓
DSI mice + D2/3 agonist	↓↓	↓	↓	↓↓

Table 2.1 Summary of changes in licking microstructures after D1 receptor agonist (A68930 or SKF38393) or D2/3 receptor agonist (ropinirole) treatment
DSI mice made fewer licks and bursts than control littermates. The D1 receptor agonist ameliorated the lick number but did not increase the burst number, and the D2 receptor agonist suppressed all the measurement results from the licking test. †The D1 agonist A68930 was effective only for DSI mice, but the D1 agonist SKF38393 was effective for both control and DSI mice.

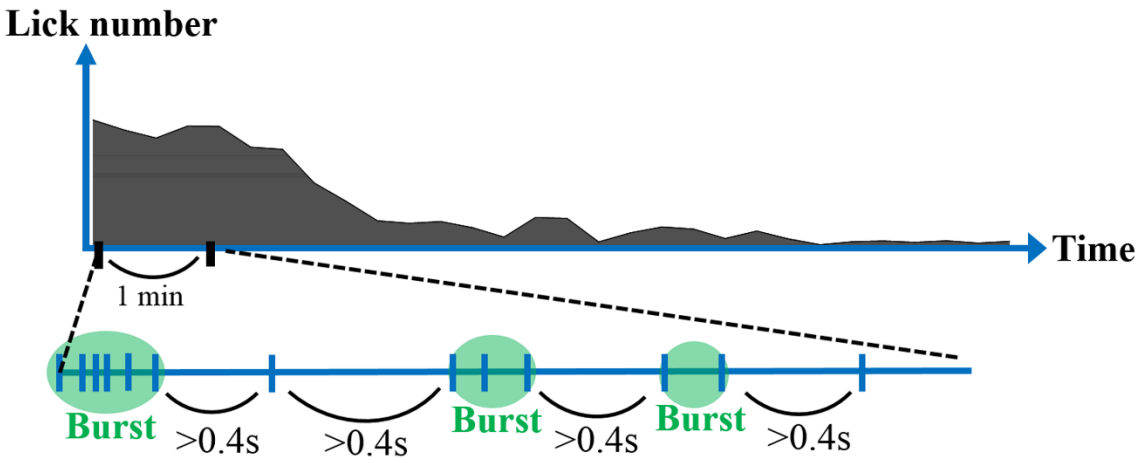

Figure 2.1 Scheme of the licking microstructure

The number of total licks represents the degree of water drinking activity and therefore is commonly used to evaluate changes in fluid ingestion and general drinking behavior. A burst is defined as continuous licking (≥ 2 licks, each vertical bar marks a lick as time progresses toward the right), in which the interval between two licks is < 0.4 s. The size of bursts represents the hedonic impact brought by the taste and reflects the orosensory positive feedback, and the intra-burst lick speed is used as an indicator of licking-associated motor control to reveal the effect on tongue movement. The number of bursts reflects the incentive motivation triggered by cues because it indicates the activation of responses and is highly affected by orosensory and post-ingestive mechanisms. To avoid the influence of consumed water, only the bursts within the first minute after the first lick were recorded.

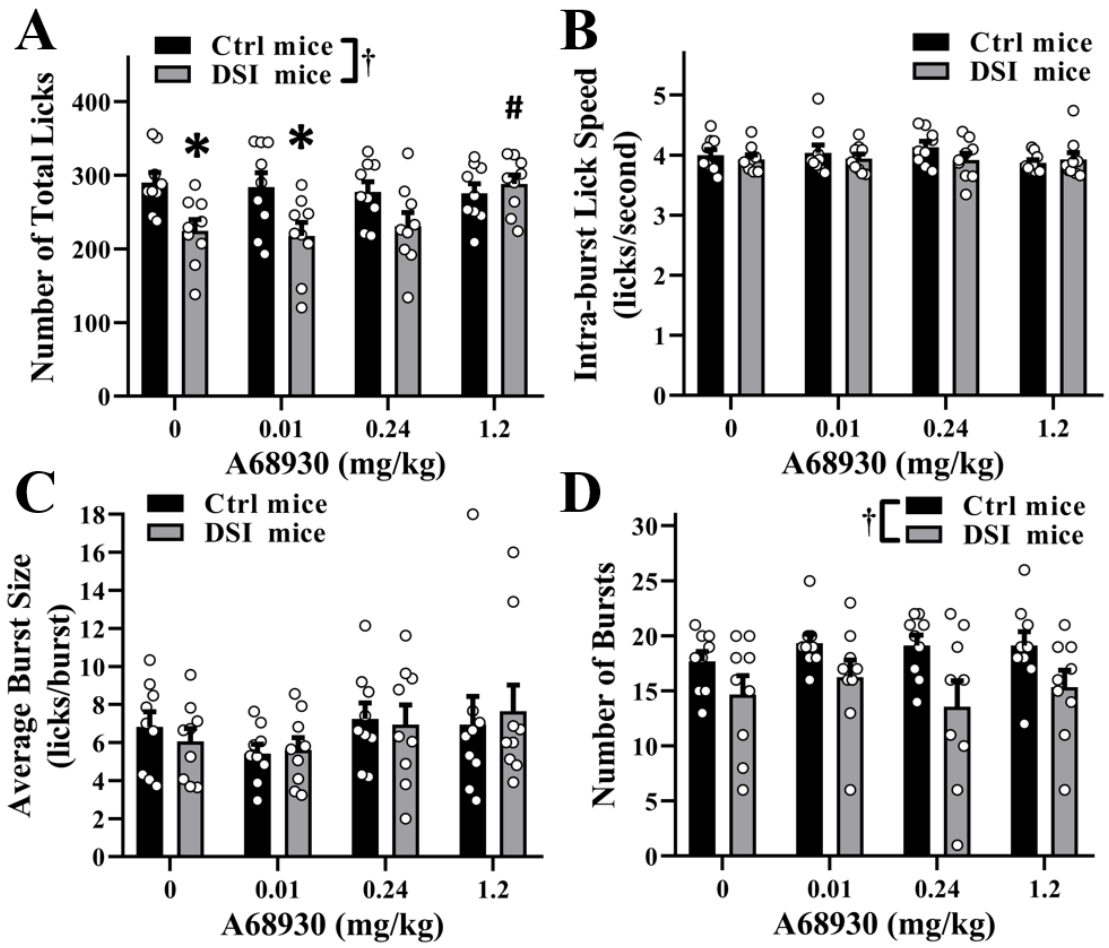

Figure 2.2 Effect of DA D1 receptor agonist, A68930, on the licking microstructure
Mice received a single injection of vehicle (saline) or A68930 (0.01 to 1.2 mg/kg), 5 min before the test session. **(A)** Comparison of the numbers of total licks after various treatments (RM-ANOVA: genotype, $p < 0.05$; genotype × dose, $p < 0.05$). After the first lick, the average lick speed within bursts **(B)**, the average size of bursts **(C)**, and the number of bursts (RM-ANOVA: genotype, $p < 0.05$) **(D)** during the first minute were recorded and compared (Ctrl, $n = 9$; DSI, $n = 9$). †$p < 0.05$ for comparison of genotype. *$p < 0.05$ compared to Ctrl mice. #$p < 0.05$ compared to vehicle treatment. Values are shown as the means ± SEMs.

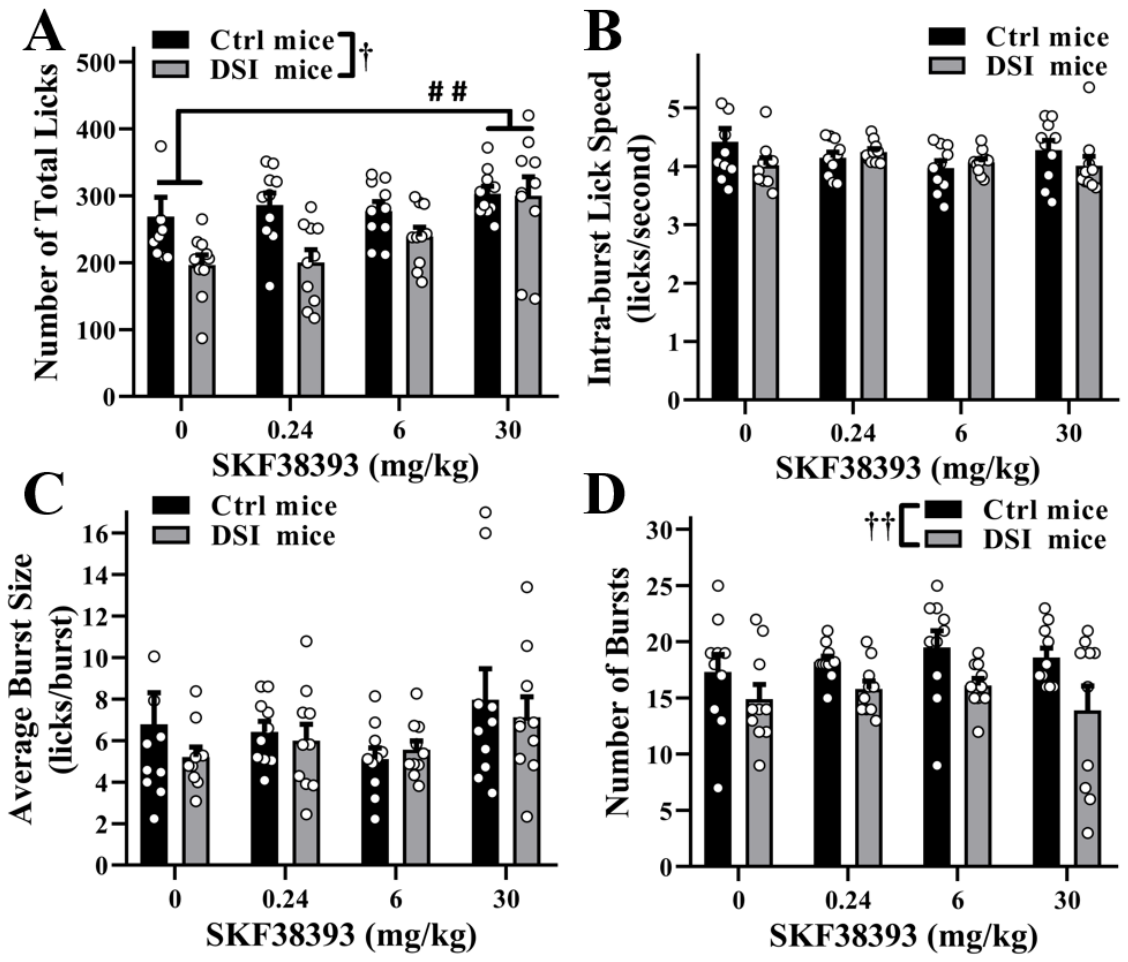

Figure 2.3 Effect of DA D1 receptor agonist, SKF38393, on the licking microstructure
Mice received a single injection of vehicle (saline) or SKF38393 (0.24 to 30 mg/kg), 5 min before the test session. **(A)** Comparison of the numbers of total licks after various treatments (RM-ANOVA: genotype, $p < 0.05$; dose, $p < 0.01$). After the first lick, the average lick speed within bursts **(B)**, the average size of bursts **(C)**, and the number of bursts (RM-ANOVA: genotype, $p < 0.01$) **(D)** during the first minute were recorded and compared (Ctrl, n =10; DSI, n = 10). †$p < 0.05$ and ††$p < 0.01$ for comparison of genotype. ##$p < 0.01$ compared to vehicle treatment. Values are shown as the means ± SEMs.

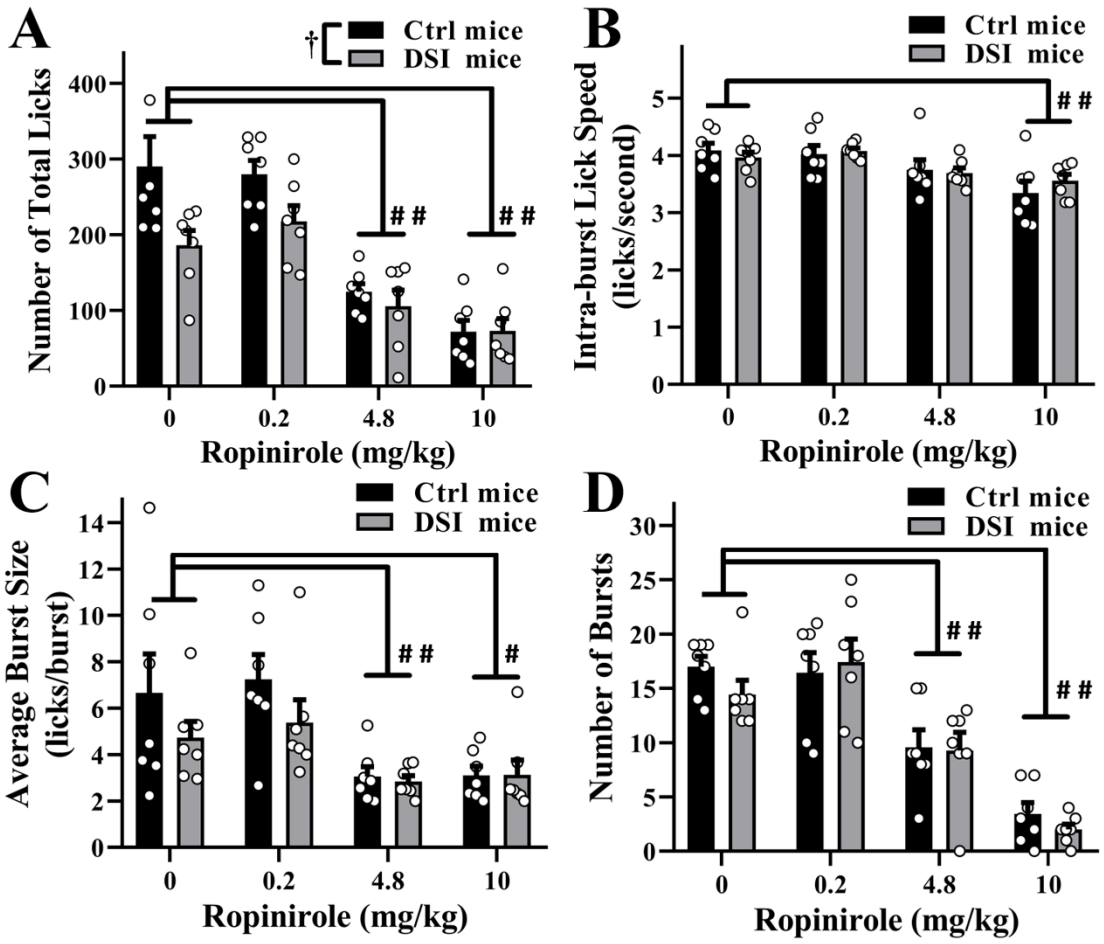

Figure 2.4 Effects of DA D2/3 receptor agonist, ropinirole, on the licking microstructure

Mice received a single injection of vehicle (saline) or ropinirole (0.2 to 10 mg/kg), 5 min before the test session. **(A)** Comparison of the numbers of total licks after various treatments (RM-ANOVA: genotype, $p < 0.05$; dose, $p < 0.01$). After the first lick, the average lick speed within bursts (RM-ANOVA: dose, $p < 0.01$] **(B)**, the average size of bursts (RM-ANOVA: dose, $p < 0.01$) **(C)**, and the number of bursts (RM-ANOVA: dose, $p < 0.01$) **(D)** during the first minute were recorded and compared (Ctrl, $n = 7$; DSI, $n = 7$). †$p < 0.05$ for comparison of genotype. #$p < 0.05$ and ##$p < 0.01$ compared to vehicle treatment. Values are shown as the means ± SEMs.

Discussion

The DA D1-like receptor antagonist SCH 23390 reduces the number of licks by rodents for various fluid rewards (water, NaCl solution, or sucrose solution) by decreasing the burst number (D'Aquila, 2010; D'Aquila *et al.*, 2012; Galistu & D'Aquila, 2012, 2013); thus the D1-like receptors were suggested to play a role in the activation of reward-associated responses. By contrast, the DA D2-like receptor antagonist raclopride reduces the number of licks by decreasing the burst size (Schneider *et al.*, 1990; Canu *et al.*, 2010; D'Aquila, 2010; D'Aquila *et al.*, 2012; Galistu & D'Aquila, 2013); therefore, the D2-like receptors were suggested to be involved in the evaluation of reward. Moreover, treatment with a D2/D3 receptor agonist, such as 7-OH-DPAT or quinpirole, suppresses licking for a sucrose solution by reducing the bout duration (Genn et al., 2003).

Effect of DA D1 receptor agonist on the licking microstructure

The findings from the D1 receptor agonist treatment showed that the decrease in the numbers of licks by DSI mice was restored by treatment with the D1 receptor agonist A68930 and that the SKF38393 treatment increased the numbers of licks in both control and DSI mice. The effects of A68930 and SKF38393 may differ because of their different drug distributions in the brain and binding selectivities, which would suggest that the effects of D1 agonists depend on how the neural circuitries are stimulated. 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 that D1 agonist treatment restored the number of licks but not the number of

bursts in DSI mice may support this, suggesting that the recovered lick number largely reflects an increase in the hedonic impact. However, previous studies in rats suggested that D1-like receptor signaling is involved in the incentive motivation (D'Aquila, 2010; D'Aquila *et al.*, 2012; Galistu & D'Aquila, 2012, 2013). This discrepancy might reflect differences in species, drugs, or administration routes. Of note, there was no significant change in burst size, which is also used as a measure of hedonic impact (D'Aquila, 2010). Therefore, more work is needed to elucidate the mechanism(s) by which D1 agonists ameliorate the water drinking behavior of DSI mice, which may involve alterations in postingestive feedback and thirst perception (Bouchaud & Bosler, 1986; Miyahara et al., 2012). Nevertheless, the restoration of the drinking behavior in DSI mice is evidence that the D1 receptor agonist A68930 may be a drug candidate to treat illnesses related to mild to moderate DA loss, including anorexia nervosa, as suggested by G. K. W. Frank (2014).

Effect of DA D2/3 receptor agonist on the licking microstructure

The D2-like receptors are crucial for the performance of acquired conditioned responses and goal-tracking behavior (Fraser et al., 2016; Lopez et al., 2015; Randall et al., 2012). In the present study, the D2/3 agonist ropinirole decreased the intra-burst lick speeds of both DSI and control mice, indicating that ropinirole impairs licking-associated motor control and reflecting the role of DA in movement (Gerfen, 1992; Puglisi-Allegra & Ventura, 2012; Salamone *et al.*, 2016). Ropinirole treatment also reduced the number and size of bursts, indicating lower incentive motivation in the mice and a decreased hedonic impact of the water reward. 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; Canu *et al.*, 2010; D'Aquila, 2010; D'Aquila *et al.*, 2012;

Galistu & D'Aquila, 2013). Overall, interfering with DA D2-like receptor signaling (by either stimulation or blockade) may suppress fluid ingestion, but the underlying cause may not be straightforward and may involve several neural mechanisms.

The findings by the DA agonist treatment (Table 2.1) may suggest the involvement of DAergic neurons in mechanisms that specifically regulate water intake, such as postingestive feedback and thirst perception (Gizowski & Bourque, 2018; Zimmerman, Leib, & Knight, 2017), in addition to the ones (motor control, hedonic impact of reward and incentive motivation) assessed by the licking microstructure analysis.

Chapter 3

Dopamine D2-like Receptor Agonist Changes Effort-based Decision-making of Mouse

本博士論文中、第三章(pp. 72-85)の部分は、European Journal of Neuroscience に掲載等の形で刊行される予定であるため、学位授与日から5年間インターネットでの公表をすることができません。

Introduction

Materials and Methods

Results

Table and Figures

Discussion

Prospects and General Discussion

Studies of reward-oriented behavior unveil how animals modulate their behaviors in response to environmental changes and their needs. Here, a new triple transgenic mouse line (i.e., DSI mouse) is developed to help elucidate the roles of DA in reward-oriented behavior by licking microstructure analysis. Specifically, the DSI mouse model enables investigations of DA signaling while avoiding the severe drawbacks of DA deletion. In fact, the performance of DSI mice nearly matched that of control littermates. However, the limited DA secretion interference has a drawback that much careful observation is required in order to reveal the behavioral difference between control and DSI mice. The DA concentrations in DSI mice were only reduced to 55% to 61% after tamoxifen administration while 46% to 52% of the DAergic neurons underwent the Cre-loxP recombination. It appears that some DAergic neurons were more insusceptible to the tamoxifen-induced Cre-loxP recombination probably due to their distance from microvessels and/or compact chromatin structure. In addition, the relatively weak activity of α-CaMKII promoter (Tg2) in DAergic neurons (Burgin *et al.*, 1990; Wang *et al.*, 2013) might restrict the expression of Tg3 and allow of sparse DA secretion.

It is reported that even with 95% loss of DA in the striatum or the NAc, rodents are able to consume minimum food to live and express almost normal goal-directed behavior (Salamone & Correa, 2002; Szczypka et al., 2001). Accordingly, the experiments with DSI mice can be further improved by slightly enhancing the DA secretion interference, and it will enlarge the difference between control and DSI mice and make it easier to observe. Firstly, the α-CaMKII promoter (Tg2) can be replaced

with a *Slc6a3* promoter by crossbreeding a transgenic mouse harboring Tg1 and Tg3 with a mouse line [STOCK-Tg(Slc6a3-tTA)2Kftnk] created by Prof. Tanaka of Keio University to enhance the transcription of tTA and then the Tg3 expression. Secondly, elevating the given concentration of tamoxifen and prolonging its consecutive treatment will increase the chance to induce Cre-loxP recombination in those DAergic neurons that are relatively far from the microvessels.

The use of different methods to examine the role of DA may provide new information and further our understanding of reward-oriented behavior. Inspired by the study by Komiyama *et al.* (2010), the licking test can be combined with functional magnetic resonance imaging in the future to study the brain regions underlying fluid ingestion. However, such studies require restriction of the movements of behaving mice (Jomura, Shintani, Sakurai, Kaneko, & Hisatsune, 2017). As restraint promotes the release of stress hormones, such as corticosterone and norepinephrine (Keim & Sigg, 1976; Grissom & Bhatnagar, 2009; Herman, 2013), it is necessary to prolong the training session in order to facilitate habituation to the experimental conditions.

Findings by this study may contribute to new treatments for illnesses related to DA loss, including anorexia nervosa, as suggested by G. K. W. Frank (2014). In chapter 1, the DSI mice show adequate capacity to consume food and water by themselves, and have no difficulty in learning a specific location or an association between location and reward. Despite the motor control impairment only under a challenging situation, DSI mice have been proved to be a useful tool for studying the role of DA in reward-oriented behavior. In chapter 2, the findings reveal that the D1 agonist A68930 ameliorates the suppression of water drinking resulting from DA loss (Table 2.1), whereas the D2/3 agonist ropinirole impedes water drinking. It may suggest the involvement of DAergic neurons in mechanisms, which may be related to

postingestive feedback and thirst perception, in addition to the ones (motor control, hedonic impact of reward and incentive motivation) assessed by the licking microstructure analysis. More discrete behavioral tests with DSI mice, which provide different rewards (e.g. sucrose, saccharine, regular chow, and high fat chow), should be helpful to ascertain the role of D1 receptor in hedonic impact.

Regarding the reward-oriented behavior, a new effort-based choice task based on licking test was developed in this study to help elucidate how an animal assesses costs and benefits of a behavior. Since the DA agonists had not yet been used in this type of study, chapter 3 examines the effect of DA agonist on the effort-based decision-making of mice. Even with 60% of DA concentration of control mice, the DSI mice are shown to be able to learn and make an effort-based decision as control mice. This finding may suggest that mild to moderate DA loss is not sufficient to impair the decision-making of mice. However, the process of decision-making in DSI mice may be more susceptible to other factors, such as aging and stress, which may influence DA receptor signaling, and this possibility can be examined by treatment of low concentration of DA antagonist, especially D2-like receptor antagonist. The treatment of DA agonist reveals that, even though the D1 agonist SKF38393 ameliorates the number of licks in DSI mice, it exerts no effect on the choice made by mice. By contrast, the D2/3 agonist ropinirole prevents mice from showing their original low-effort bias regardless of DA status (Table 3.1). This result indicates an important role of D2-like receptors in decision-making of mice during water drinking behavior. Perhaps a series of effort-based choice tasks of chapter 3 that provides different concentrations of reward can help to further discriminate between the role of DA in reward evaluation and the role of DA in effort evaluation.

References

Baldo, B. A., & Kelley, A. E. (2007). Discrete neurochemical coding of distinguishable motivational processes: Insights from nucleus accumbens control of feeding. *Psychopharmacology (Berl)*, *191*(3), 439–459. https://doi.org/10.1007/s00213-007-0741-z

Berridge, K. C. (1996). Food Reward: Brain Substrates of Wanting and Liking. *Neuroscience & Biobehavioral Reviews*, *20*(1), 1–25. https://doi.org/10.1016/0149-7634(95)00033-B

Berridge, K. C. (2018). Evolving Concepts of Emotion and Motivation. *Frontiers in Psychology*, *9*(1647), 1–20. https://doi.org/10.3389/fpsyg.2018.01647

Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol.*, *9*(1), 65–73. https://doi.org/10.1016/j.coph.2008.12.014.

Bindra, D. (1978). How adaptive behavior is produced: A perceptual-motivational alternative to response reinforcements. *Behavioral and Brain Sciences*, *1*, 41–91. https://doi.org/10.1017/S0140525X00059380

Borisovska, M., Bensen, A., Chong, G., & Westbrook, G. L. (2013). Distinct modes of dopamine and GABA release in a dual transmitter neuron. *J Neurosci.*, *33*(5), 1790–1796. https://doi.org/10.1523/JNEUROSCI.4342-12.2013

Bouchaud, C., & Bosler, O. (1986). The Circumventricular Organs of the Mammalian Brain with Special Reference to Monoaminergic Innervation. *International Review of Cytology*, *105*, 283–327. https://doi.org/10.1016/S0074-7696(08)61066-9

Breslin, P. A. S. (2013). An evolutionary perspective on food and human taste. *Current Biology*, *23*(9), R409–R418. https://doi.org/10.1016/j.cub.2013.04.010

Burgin, K. E., Waxham, M. N., Rickling, S., Westgate, S. A., Mobley, W. C., & Kelly, P. T. (1990). In situ hybridization histochemistry of Ca2+/calmodulin-dependent protein kinase in developing rat brain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *10*(6), 1788–1798. https://doi.org/10.1523/JNEUROSCI.10-06-01788.1990

Campbell, B. A. (1960). Effects of water deprivation on random activity. *Journal of Comparative and Physiological Psychology*, *53*(3), 240–241. https://doi.org/10.1037/h0044975

Cannon, C. M., Abdallah, L., Tecott, L. H., During, M. J., & Palmiter, R. D. (2004). Dysregulation of striatal dopamine signaling by amphetamine inhibits feeding by hungry mice. *Neuron*, *44*(3), 509–520. https://doi.org/10.1016/j.neuron.2004.10.009

Cannon, C. M., & Palmiter, R. D. (2003). Reward without dopamine. *The Journal of Neuroscience*, *23*(34), 10827–10831. https://doi.org/23/34/10827

Canu, M. E., Carta, D., Murgia, E., Serra, G., & D'Aquila, P. S. (2010). Dopamine on D2-like receptors is involved in reward evaluation in water-deprived rats licking for NaCl and water. *Pharmacology Biochemistry and Behavior*, *96*(2), 194–197. https://doi.org/10.1016/j.pbb.2010.05.004

Clark, J. J., Collins, A. L., Sanford, C. A., & Phillips, P. E. M. (2013). Dopamine encoding of Pavlovian incentive stimuli diminishes with extended training. *J Neurosci.*, *33*(8), 3526–3532. https://doi.org/10.1523/JNEUROSCI.5119-12.2013

Cousins, M. S., Atherton, A., Turner, L., & Salamone, J. D. (1996). Nucleus accumbens dopamine depletions alter relative response allocation in a T-maze cost/benefit

task. *Behavioural Brain Research*, *74*(1–2), 189–197. https://doi.org/10.1016/0166-4328(95)00151-4

Cousins, M. S., Wei, W., & Salamone, J. D. (1994). Pharmacological characterization of performance on a concurrent lever pressing/feeding choice procedure: effects of dopamine antagonist, cholinomimetic, sedative and stimulant drugs. *Psychopharmacology*, *116*(4), 529–537. https://doi.org/10.1007/BF02247489

D'Aquila, P. S. (2010). Dopamine on D2-like receptors "reboosts" dopamine D1-like receptor-mediated behavioural activation in rats licking for sucrose. *Neuropharmacology*, *58*(7), 1085–1096. https://doi.org/10.1016/j.neuropharm.2010.01.017

D'Aquila, P. S., & Galistu, A. (2017). Within-session decrement of the emission of licking bursts following reward devaluation in rats licking for sucrose. *PLoS ONE*, *12*(5), 1–25. https://doi.org/10.1371/journal.pone.0177705

D'Aquila, P. S., Rossi, R., Rizzi, A., & Galistu, A. (2012). Possible role of dopamine D1-like and D2-like receptors in behavioural activation and "contingent" reward evaluation in sodium-replete and sodium-depleted rats licking for NaCl solutions. *Pharmacology Biochemistry and Behavior*, *101*(1), 99–106. https://doi.org/10.1016/j.pbb.2011.12.004

Danna, C. L., & Elmer, G. I. (2010). Disruption of conditioned reward association by typical and atypical antipsychotics. *Pharmacol Biochem Behav.*, *96*(1), 40–47. https://doi.org/10.1016/j.pbb.2010.04.004

Darvas, M., & Palmiter, R. D. (2009). Restriction of dopamine signaling to the dorsolateral striatum is sufficient for many cognitive behaviors. *Proceedings of the National Academy of Sciences*, *106*(34), 14664–14669. https://doi.org/10.1073/pnas.0907299106

Dastugue, A., Merlin, J. F., Maquart, G., Bernard, A., & Besnard, P. (2018). A New Method for Studying Licking Behavior Determinants in Rodents: Application to Diet-Induced Obese Mice. *Obesity*, *26*(12), 1905–1914. https://doi.org/10.1002/oby.22342

Davies, J. R., Humby, T., Dwyer, D. M., Garfield, A. S., Furby, H., Wilkinson, L. S., Wells, T., & Isles, A. R. (2015). Calorie seeking, but not hedonic response, contributes to hyperphagia in a mouse model for Prader-Willi syndrome. *European Journal of Neuroscience*, *42*(4), 1–9. https://doi.org/10.1111/ejn.12972

Davis, J. D. (1989). The Microstructure of Ingestive Behavior. *Annals of the New York Academy of Sciences*, *575*, 106–119. https://doi.org/10.1111/j.1749-6632.1989.tb53236.x

Davis, J. D., & Smith, G. P. (1992). Analysis of the Microstructure of the Rhythmic Tongue Movements of Rats Ingesting Maltose and Sucrose Solutions. *Behavioral Neuroscience*, *106*(1), 217–228. https://doi.org/10.1037/0735-7044.106.1.217

Dickinson, A., Smith, J., & Mirenowicz, J. (2000). Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behavioral Neuroscience*, *114*, 468–483. https://doi.org/10.1037/0735-7044.114.3.468

Dotson, C. D., Geraedts, M. C. P., & Munger, S. D. (2013). Peptide regulators of peripheral taste function. *Seminars in Cell and Developmental Biology*, *24*(3), 232–239. https://doi.org/10.1016/j.semcdb.2013.01.004

Dwyer, D. M. (2012). Licking and liking: The assessment of hedonic responses in rodents. *Quarterly Journal of Experimental Psychology*, *65*(3), 371–394. https://doi.org/10.1080/17470218.2011.652969

Fay, R. A., & Norgren, R. (1997). Identification of rat brainstem multisynaptic connections to the oral motor nuclei using pseudorabies virus III. Lingual muscle

motor systems. *Brain Research Reviews*, *25*(3), 291–311. https://doi.org/10.1016/S0165-0173(97)00028-3

Flagel, S. B., & Robinson, T. E. (2017). Neurobiological basis of individual variation in stimulus-reward learning. *Current Opinion in Behavioral Sciences*, *13*, 178–185. https://doi.org/10.1016/j.cobeha.2016.12.004

Flood, D. G., & Coleman, P. D. (1988). Neuron numbers and sizes in aging brain: Comparisons of human, monkey, and rodent data. *Neurobiology of Aging*, *9*(5–6), 453–463. https://doi.org/10.1016/S0197-4580(88)80098-8

Floresco, S. B. (2015). The Nucleus Accumbens: An Interface between Cognition, Emotion, and Action. *Annu. Rev. Psychol.*, *66*, 25–52. https://doi.org/10.1146/annurev-psych-010213-115159

Floresco, S. B., St.Onge, J. R., Ghods-Sharifi, S., & Winstanley, C. A. (2008). Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. *Cognitive, Affective and Behavioral Neuroscience*, *8*(4), 375–389. https://doi.org/10.3758/CABN.8.4.375

Frank, G. K. W. (2014). Could Dopamine Agonists Aid in Drug Development for Anorexia Nervosa? *Frontiers in Nutrition*, *1*(19), 1–9. https://doi.org/10.3389/fnut.2014.00019

Fraser, K. M., Haight, J. L., Gardner, E. L., & Flagel, S. B. (2016). Examining the role of dopamine D2 and D3 receptors in Pavlovian conditioned approach behaviors. *Behavioural Brain Research*, *305*, 87–99. https://doi.org/10.1016/j.bbr.2016.02.022

Friedemann, M. N., & Gerhardt, G. A. (1992). Regional effects of aging on dopaminergic function in the Fischer-344 rat. *Neurobiology of Aging*, *13*(2), 325–332. https://doi.org/10.1016/0197-4580(92)90046-Z

Galistu, A., & D'Aquila, P. S. (2012). Effect of the dopamine D1-like receptor antagonist SCH 23390 on the microstructure of ingestive behaviour in water-deprived rats licking for water and NaCl solutions. *Physiology and Behavior*, *105*(2), 230–233. https://doi.org/10.1016/j.physbeh.2011.08.006

Galistu, A., & D'Aquila, P. S. (2013). Dopamine on D2-like receptors "reboosts" dopamine D1-like receptor-mediated behavioural activation in rats licking for a isotonic NaCl solution. *Psychopharmacology*, *229*(2), 357–366. https://doi.org/10.1007/s00213-013-3110-0

Genn, R. F., Higgs, S., & Cooper, S. J. (2003). The effects of 7-OH-DPAT, quinpirole and raclopride on licking for sucrose solutions in the non-deprived rat. *Behavioural Pharmacology*, *14*(8), 609–617. https://doi.org/10.1097/01.fbp.0000104032.08123.4c

Gerfen, C. R. (1992). The neostriatal mosaic: multiple levels of compartmental organization. *Trends in Neurosciences*, *15*(4), 133–139. https://doi.org/10.1016/0166-2236(92)90355-C

Gizowski, C., & Bourque, C. W. (2018). The neural basis of homeostatic and anticipatory thirst. *Nature Reviews Nephrology*, *14*(1), 11–25. https://doi.org/10.1038/nrneph.2017.149

Goltstein, P. M., Reinert, S., Glas, A., Bonhoeffer, T., & Hübener, M. (2018). Food and water restriction lead to differential learning behaviors in a head-fixed two-choice visual discrimination task for mice. *PLoS ONE*, *13*(9), e0204066. https://doi.org/10.1371/journal.pone.0204066

Gore, B. B., Miller, S. M., Jo, Y. S., Baird, M. A., Hoon, M., Sanford, C. A., Hunker, A., Lu, W., Wong, R. O., & Zweifel, L. S. (2017). Roundabout receptor 2 maintains inhibitory control of the adult midbrain. *ELife*, *6*, 1–20.

https://doi.org/10.7554/eLife.23858

Gramling, S. E., & Fowler, S. C. (1986). Some effects of pimozide and of shifts in sucrose concentration on lick rate, duration, and interlick interval. *Pharmacology, Biochemistry and Behavior*, *25*(1), 219–222. https://doi.org/10.1016/0091-3057(86)90256-X

Gramling, S. E., Fowler, S. C., & Collins, K. R. (1984). Some effects of pimozide on nondeprived rats licking sucrose solutions in an anhedonia paradigm. *Pharmacology Biochemistry and Behavior*, *21*(4), 617–624. https://doi.org/10.1016/S0091-3057(84)80047-7

Grissom, N., & Bhatnagar, S. (2009). Habituation to repeated stress: get used to it. *Neurobiol Learn Mem.*, *92*(2), 215–224. https://doi.org/10.1016/j.nlm.2008.07.001

Halbout, B., Marshall, A. T., Azimi, A., Liljeholm, M., Mahler, S.V., Wassum, K. M., & Ostlund, S. B. (2019). Mesolimbic dopamine projections mediate cue-motivated reward seeking but not reward retrieval in rats. *ELife*, *8*, e43551. https://doi.org/10.7554/eLife.43551

Hauber, W., & Sommer, S. (2009). Prefrontostriatal circuitry regulates effort-related decision making. *Cerebral Cortex*, *19*(10), 2240–2247. https://doi.org/10.1093/cercor/bhn241

Herman, J. P. (2013). Neural control of chronic stress adaptation. *Frontiers in Behavioral Neuroscience*, *7*(61), 1–12. https://doi.org/10.3389/fnbeh.2013.00061

Higgs, S., & Cooper, S. J. (1998). Evidence for early opioid modulation of licking responses to sucrose and Intralipid: A microstructural analysis in the rat. *Psychopharmacology*, *139*(4), 342–355. https://doi.org/10.1007/s002130050725

Holman, G. L. (1969). Intragastric reinforcement effect. *Journal of Comparative and*

Physiological Psychology, *69*, 432–441. https://doi.org/10.1037/h0028233

Hosking, J. G., Floresco, S. B., & Winstanley, C. A. (2015). Dopamine antagonism decreases willingness to expend physical, but not cognitive, effort: A comparison of two rodent cost/benefit decision-making tasks. *Neuropsychopharmacology*, *40*(4), 1005–1015. https://doi.org/10.1038/npp.2014.285

Jin, X., & Costa, R. M. (2010). Start/stop signals emerge in nigrostriatal circuits during sequence learning. *Nature*, *466*(7305), 457–462. https://doi.org/10.1038/nature09263

Johnson, A. W. (2018). Characterizing ingestive behavior through licking microstructure: Underlying neurobiology and its use in the study of obesity in animal models. *Int J Dev Neurosci.*, *64*, 38–47. https://doi.org/10.1016/j.ijdevneu.2017.06.012

Johnson, A. W., Jaaro-Peled, H., Shahani, N., Sedlak, T. W., Zoubovsky, S., Burruss, D., Emiliani, F., Sawa, A., & Gallagher, M. (2013). Cognitive and motivational deficits together with prefrontal oxidative stress in a mouse model for neuropsychiatric illness. *Proceedings of the National Academy of Sciences*, *110*(30), 12462–12467. https://doi.org/10.1073/pnas.1307925110

Johnson, A. W., Sherwood, A., Smith, D. R., Wosiski-Kuhn, M., Gallagher, M., & Holland, P. C. (2010). An analysis of licking microstructure in three strains of mice. *Appetite*, *54*(2), 320–330. https://doi.org/10.1016/j.appet.2009.12.007

Jomura, N., Shintani, T., Sakurai, K., Kaneko, J., & Hisatsune, T. (2017). Mouse BOLD fMRI imaging during operant learning at ultra-high field (14 T). *Proc. Intl. Soc. Mag. Reson. Med.*, *25*, 5365.

Kao, K.-C., & Hisatsune, T. (2019). Differential effects of dopamine D1-like and D2-like receptor agonists on water drinking behaviour under thirsty conditions in

mice with reduced dopamine secretion. *European Journal of Neuroscience*. https://doi.org/10.1111/ejn.14568

Keim, K. L., & Sigg, E. B. (1976). Physiological and biochemical concomitants of restraint stress in rats. *Pharmacology, Biochemistry and Behavior*, *4*(3), 289–297. https://doi.org/10.1016/0091-3057(76)90244-6

Kim, D. S., Palmiter, R. D., Cummins, A., & Gerfen, C. R. (2006). Reversal of supersensitive striatal dopamine D1 receptor signaling and extracellular signal-regulated kinase activity in dopamine-deficient mice. *Neuroscience*, *137*(4), 1381–1388. https://doi.org/10.1016/j.neuroscience.2005.10.054

Kim, D. S., Szczypka, M. S., & Palmiter, R. D. (2000). Dopamine-deficient mice are hypersensitive to dopamine receptor agonists. *Journal of Neuroscience*, *20*(12), 4405–4413. https://doi.org/10.1523/jneurosci.20-12-04405.2000

Komiyama, T., Sato, T. R., Oconnor, D. H., Zhang, Y. X., Huber, D., Hooks, B. M., Gabitto, M., & Svoboda, K. (2010). Learning-related fine-scale specificity imaged in motor cortex circuits of behaving mice. *Nature*, *464*(7292), 1182–1186. https://doi.org/10.1038/nature08897

Kosheleff, A. R., Araki, J., Hsueh, J., Le, A., Quizon, K., Ostlund, S. B., Maidment, N. T., & Murphy, N. P. (2018). Pattern of access determines influence of junk food diet on cue sensitivity and palatability. *Appetite*, *123*, 135–145. https://doi.org/10.1016/j.appet.2017.12.009

Levesque, M., Bedard, M. A., Courtemanche, R., Tremblay, P. L., Scherzer, P., & Blanchet, P. J. (2007). Raclopride-induced motor consolidation impairment in primates: Role of the dopamine type-2 receptor in movement chunking into integrated sequences. *Experimental Brain Research*, *182*, 499–508. https://doi.org/10.1007/s00221-007-1010-4

Lopez, J. C., Karlsson, R. M., & O'Donnell, P. (2015). Dopamine D2 Modulation of Sign and Goal Tracking in Rats. *Neuropsychopharmacology*, *40*(9), 2096–2102. https://doi.org/10.1038/npp.2015.68

Mai, B., Sommer, S., & Hauber, W. (2012). Motivational states influence effort-based decision making in rats: The role of dopamine in the nucleus accumbens. *Cognitive, Affective and Behavioral Neuroscience*, *12*(1), 74–84. https://doi.org/10.3758/s13415-011-0068-4

Mangel, M., & Clark, C. W. (1986). Towards a unified foraging theory. *Ecology*, *67*(5), 1127–1138. https://doi.org/10.2307/1938669

McClure, S. M., Daw, N. D., & Montague, P. R. (2003). A computational substrate for incentive salience. *Trends in Neurosciences*, *26*(8), 423–428. https://doi.org/10.1016/S0166-2236(03)00177-2

McNamara, J. M., & Houston, A. I. (1986). The Common Currency for Behavioral Decisions. *The American Naturalist*, *127*(3), 358–378. https://doi.org/10.1086/284489

Mendez, I. A., Maidment, N. T., & Murphy, N. P. (2016). Parsing the hedonic and motivational influences of nociceptin on feeding using licking microstructure analysis in mice. *Behavioural Pharmacology*, *27*(6), 516–527. https://doi.org/10.1097/FBP.0000000000000240

Mendez, I. A., Ostlund, S. B., Maidment, N. T., & Murphy, N. P. (2015). Involvement of Endogenous Enkephalins and β-Endorphin in Feeding and Diet-Induced Obesity. *Neuropsychopharmacology*, *40*(9), 2103–2112. https://doi.org/10.1038/npp.2015.67

Mennella, J. A., Daniels, L. M., & Reiter, A. R. (2017). Learning to like vegetables during breastfeeding: A randomized clinical trial of lactating mothers and infants.

American Journal of Clinical Nutrition, *106*(1), 67–76. https://doi.org/10.3945/ajcn.116.143982

Miyahara, N., Ono, K., Hitomi, S., Hirase, M., & Inenaga, K. (2012). Dopamine modulates neuronal excitability pre- and post-synaptically in the rat subfornical organ. *Brain Research*, *1447*, 44–52. https://doi.org/10.1016/j.brainres.2012.01.063

Nakashiba, T., Young, J. Z., McHugh, T. J., Buhl, D. L., & Tonegawa, S. (2008). Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning. *Science.*, *319*(5867), 1260–1264. https://doi.org/10.1126/science.1151120

Nicolaidis, S., & Rowland, N. (1975). Regulatory drinking in rats with permanent access to a bitter fluid source. *Physiology and Behavior*, *14*, 819–824. https://doi.org/10.1016/0031-9384(75)90076-1

Nunes, E. J., Randall, P. A., Hart, E. E., Freeland, C., Yohn, S. E., Baqi, Y., Muller, C. E., Lopez-Cruz, L., Correa, M., & Salamone, J. D. (2013). Effort-Related Motivational Effects of the VMAT-2 Inhibitor Tetrabenazine: Implications for Animal Models of the Motivational Symptoms of Depression. *Journal of Neuroscience*, *33*(49), 19120–19130. https://doi.org/10.1523/JNEUROSCI.2730-13.2013

Ostlund, S. B., Kosheleff, A., Maidment, N. T., & Murphy, N. P. (2013). Decreased consumption of sweet fluids in mu opioid receptor knockout mice: A microstructural analysis of licking behavior. *Psychopharmacology*, *229*(1), 105–113. https://doi.org/10.1007/s00213-013-3077-x.

Ostlund, S. B., Wassum, K. M., Murphy, N. P., Balleine, B. W., & Maidment, N. T. (2011). Extracellular Dopamine Levels in Striatal Subregions Track Shifts in

Motivation and Response Cost During Instrumental Conditioning. *J Neurosci.*, *31*(1), 200–207. https://doi.org/10.1523/JNEUROSCI.4759-10.2011

Ostlund, S. B., & Maidment, N. T. (2012). Dopamine receptor blockade attenuates the general incentive motivational effects of noncontingently delivered rewards and reward-paired cues without affecting their ability to bias action selection. *Neuropsychopharmacology*, *37*, 508–519. https://doi.org/10.1038/npp.2011.217

Palmiter, R. D. (2008). Dopamine Signaling in the Dorsal Striatum Is Essential for Motivated Behaviors: Lessons from Dopamine-deficient Mice. *Annals of the New York Academy of Sciences*, *1129*(1), 35–46. https://doi.org/10.1196/annals.1417.003

Peciña, S., & Berridge, K. C. (2013). Dopamine or opioid stimulation of nucleus accumbens similarly amplify cue-triggered "wanting" for reward: Entire core and medial shell mapped as substrates for PIT enhancement. *European Journal of Neuroscience*, *37*(9), 1529–1540. https://doi.org/10.1111/ejn.12174

Peter, D., Liu, Y., Sternini, C., deGiorgio, R., Brecha, N., & Edwards, R. (1995). Differential expression of two vesicular monoamine transporters. *The Journal of Neuroscience*, *15*(9), 6179–6188. https://doi.org/10.1523/JNEUROSCI.15-09-06179.1995

Petrovich, G. D. (2011). Learning and the motivation to eat: Forebrain circuitry. *Physiology and Behavior*, *104*(4), 582–589. https://doi.org/10.1016/j.physbeh.2011.04.059

Puglisi-Allegra, S., & Ventura, R. (2012). Prefrontal/accumbal catecholamine system processes emotionally driven attribution of motivational salience. *Reviews in the Neurosciences*, *23*(5–6), 509–526. https://doi.org/10.1515/revneuro-2012-0076

Randall, P. A., Lee, C. A., Nunes, E. J., Yohn, S. E., Nowak, V., Khan, B., …Salamone,

J. D. (2014). The VMAT-2 inhibitor tetrabenazine affects effort-related decision making in a progressive ratio/chow feeding choice task: Reversal with antidepressant drugs. *PLoS ONE*, *9*(6), e99320. https://doi.org/10.1371/journal.pone.0099320

Randall, P. A., Pardo, M., Nunes, E. J., López Cruz, L., Vemuri, V. K., Makriyannis, A., Baqi, Y., Müller, C. E., Correa, M., & Salamone, J. D. (2012). Dopaminergic Modulation of Effort-Related Choice Behavior as Assessed by a Progressive Ratio Chow Feeding Choice Task: Pharmacological Studies and the Role of Individual Differences. *PLoS ONE*, *7*(10), e47934. https://doi.org/10.1371/journal.pone.0047934

Robinson, S., Sandstrom, S. M., Denenberg, V. H., & Palmiter, R. D. (2005). Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. *Behavioral Neuroscience*, *119*(1), 5–15. https://doi.org/10.1037/0735-7044.119.1.5

Robinson, M. J. F., & Berridge, K. C. (2013). Instant transformation of learned repulsion into motivational 'wanting.' *Curr Biol.*, *23*(4), 282–289. https://doi.org/10.1016/j.cub.2013.01.016

Robles, C. F., & Johnson, A. W. (2017). Disruptions in effort-based decision-making and consummatory behavior following antagonism of the dopamine D2 receptor. *Behavioural Brain Research*, *320*, 431–439. https://doi.org/10.1016/j.bbr.2016.10.043

Salamone, J. D., Arizzi, M., Sandoval, M., Cervone, K., & Aberman, J. (2002). Dopamine antagonists alter response allocation but do not suppress appetite for food in rats: Contrast between the effects of SKF 83566, raclopride, and fenfluramine on a concurrent choice task. *Psychopharmacology*, *160*(4), 371–380.

https://doi.org/10.1007/s00213-001-0994-x

Salamone, J. D., & Correa, M. (2002). Motivational views of reinforcement: Implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behavioural Brain Research, 137,* 3–25. https://doi.org/10.1016/S0166-4328(02)00282-6

Salamone, J. D., Correa, M., Yang, J.-H., Rotolo, R., & Presby, R. (2018). Dopamine, Effort-Based Choice, and Behavioral Economics: Basic and Translational Research. *Frontiers in Behavioral Neuroscience, 12*(52), 1–13. https://doi.org/10.3389/fnbeh.2018.00052

Salamone, J. D., Cousins, M. S., & Bucher, S. (1994). Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behavioural Brain Research, 65*(2), 221–229. https://doi.org/10.1016/0166-4328(94)90108-2

Salamone, J. D., Pardo, M., Yohn, S. E., López-Cruz, L., Sanmiguel, N., & Correa, M. (2016). Mesolimbic dopamine and the regulation of motivated behavior. *Current Topics in Behavioral Neurosciences, 27,* 231–257. https://doi.org/10.1007/7854_2015_383

Salamone, J. D., Steinpreis, R. E., McCullough, L. D., Smith, P., Grebel, D., & Mahan, K. (1991). Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. *Psychopharmacology, 104*(4), 515–521. https://doi.org/10.1007/BF02245659

Saunders, B. T., & Robinson, T. E. (2012). The role of dopamine in the accumbens core in the expression of Pavlovian conditioned responses. *Eur J Neurosci., 36*(4), 2521–2532. https://doi.org/10.1111/j.1460-9568.2012.08217.x.

Schiavo, G., Benfenatit, F., Poulain, B., Rossetto, O., Laureto, P. P.de, DasGupta, B. R., & Montecucco, C. (1992). Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature*, *359*(6398), 832–835. https://doi.org/10.1038/359832a0

Schneider, L. H., Davis, J. D., Watson, C. A., & Smith, G. P. (1990). Similar effect of raclopride and reduced sucrose concentration on the microstructure of sucrose sham feeding. *European Journal of Pharmacology*, *186*(1), 61–70. https://doi.org/10.1016/0014-2999(90)94060-B

Schoch, S., Deák, F., Königstorfer, A., Mozhayeva, M., Sara, Y., Südhof, T. C., & Kavalali, E. T. (2001). SNARE Function Analyzed in Synaptobrevin/VAMP Knockout Mice. *Science.*, *294*(5544), 1117–1122. https://doi.org/10.1126/science.1064335

Schriever, S. C., Zimprich, A., Pfuhlmann, K., Baumann, P., Giesert, F., Klaus, V., …Pfluger, P. T. (2017). Alterations in neuronal control of body weight and anxiety behavior by glutathione peroxidase 4 deficiency. *Neuroscience*, *357*, 241–254. https://doi.org/10.1016/j.neuroscience.2017.05.050

Schultz, W., Stauffer, W. R., & Lak, A. (2017). The phasic dopamine signal maturing: from reward via behavioural activation to formal economic utility. *Current Opinion in Neurobiology*, *43*, 139–148. https://doi.org/10.1016/j.conb.2017.03.013

Schweimer, J., & Hauber, W. (2006). Dopamine D1 receptors in the anterior cingulate cortex regulate effort-based decision making. *Learning and Memory*, *13*(6), 777–782. https://doi.org/10.1101/lm.409306

Sharpe, M. J., Chang, C. Y., Liu, M. A., Batchelor, H. M., Mueller, L. E., Jones, J. L., Niv, Y., & Schoenbaum, G. (2017). Dopamine transients are sufficient and

necessary for acquisition of model-based associations. *Nature Neuroscience*, *20*, 735–742. https://doi.org/10.1038/nn.4538

Singer, B. F., Guptaroy, B., Austin, C. J., Wohl, I., Lovic, V., Seiler, J. L., Vaughan, R. A., Gnegy, M. E., Robinson, T. E., & Aragona, B. J. (2016). Individual variation in incentive salience attribution and accumbens dopamine transporter expression and function. *Eur J Neurosci*, *43*(5), 662–670. https://doi.org/10.1111/ejn.13134

Sink, K. S., Vemuri, V. K., Olszewska, T., Makriyannis, A., & Salamone, J. D. (2008). Cannabinoid CB1 antagonists and dopamine antagonists produce different effects on a task involving response allocation and effort-related choice in food-seeking behavior. *Psychopharmacology*, *196*(4), 565–574. https://doi.org/10.1007/s00213-007-0988-4

Skinner, B. F. (1938). *The Behavior of Organisms: An Experimental Analysis*. New York: Appleton-Century-Crofts.

Smith, G. P. (2001). John Davis and the meanings of licking. *Appetite*, *36*(1), 84–92. https://doi.org/10.1006/appe.2000.0371

Sommer, S., Danysz, W., Russ, H., Valastro, B., Flik, G., & Hauber, W. (2014). The dopamine reuptake inhibitor MRZ-9547 increases progressive ratio responding in rats. *International Journal of Neuropsychopharmacology*, *17*(12), 2045–2056. https://doi.org/10.1017/S1461145714000996

Sotak, B. N., Hnasko, T. S., Robinson, S., Kremer, E. J., & Palmiter, R. D. (2005). Dysregulation of dopamine signaling in the dorsal striatum inhibits feeding. *Brain Research*, *1061*(2), 88–96. https://doi.org/10.1016/j.brainres.2005.08.053

Steinberg, E. E., Boivin, J. R., Saunders, B. T., Witten, I. B., Deisseroth, K., & Janak, P. H. (2014). Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. *PLoS ONE*, *9*,

e94771. https://doi.org/10.1371/journal.pone.0094771

Szczypka, M. S., Kwok, K., Brot, M. D., Marck, B. T., Matsumoto, A. M., Donahue, B. A., & Palmiter, R. D. (2001). Dopamine Production in the Caudate Putamen Restores Feeding in Dopamine-Deficient Mice. *Neuron*, *30*, 819–828. Retrieved https://doi.org/10.1016/s0896-6273(01)00319-1

Szczypka, M. S., Rainey, M. A., Kim, D. S., Alaynick, W. A., Marck, B. T., Matsumoto, A. M., & Palmiter, R. D. (1999). Feeding behavior in dopamine-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 12138–12143. https://doi.org/10.1073/pnas.96.21.12138

Taha, S., & Fields, H. L. (2005). Encoding of Palatability and Appetitive Behaviors by Distinct Neuronal Populations in the Nucleus Accumbens. *Journal of Neuroscience*, *25*(5), 1193–1202. https://doi.org/10.1523/jneurosci.3975-04.2005

Thomas, S. A., Matsumoto, A. M., & Palmiter, R. D. (1995). Noradrenaline is essential for mouse fetal development. *Nature*, *374*(6523), 643–646. https://doi.org/10.1038/374643a0

Toates, F. (2012). Operant Behavior. In N. M.Seel (Ed.), *Encyclopedia of the Sciences of Learning*. Boston, MA: Springer US.

Toates, F. M. (1986). *Motivational Systems*. Cambridge: Cambridge University Press.

Tonelli, L., & Chiaraviglio, E. (1995). Dopaminergic neurons in the zona incerta modulates ingestive behavior in rats. *Physiology and Behavior*, *58*(4), 725–729. https://doi.org/10.1016/0031-9384(95)00128-6

Travers, J. B., Dinardo, L. A., & Karimnamazi, H. (1997). Motor and premotor mechanisms of licking. *Neuroscience and Biobehavioral Reviews*, *21*(5), 631–647. https://doi.org/10.1016/S0149-7634(96)00045-0

Tritsch, N. X., Ding, J. B., & Sabatini, B. L. (2012). Dopaminergic neurons inhibit

striatal output via non-canonical release of GABA. *Nature*, *490*(7419), 262–266. https://doi.org/10.1038/nature11466

Tsutsui-Kimura, I., Natsubori, A., Mori, M., Kobayashi, K., Drew, M. R., deKerchove d'Exaerde, A., Mimura, M., & Tanaka, K. F. (2017). Distinct Roles of Ventromedial versus Ventrolateral Striatal Medium Spiny Neurons in Reward-Oriented Behavior. *Current Biology*, *27*(19), 3042–3048. https://doi.org/10.1016/j.cub.2017.08.061

Uematsu, A., Tsurugizawa, T., Kitamura, A., Ichikawa, R., Iwatsuki, K., Uneyama, H., & Torii, K. (2011). Evaluation of the "liking" and "wanting" properties of umami compound in rats. *Physiology and Behavior*, *102*(5), 553–558. https://doi.org/10.1016/j.physbeh.2011.01.005

Wang, X., Zhang, C., Szábo, G., & Sun, Q.-Q. (2013). Distribution of CaMKIIα expression in the brain in vivo, studied by CaMKIIα-GFP mice. *Brain Research*, *1518*, 9–25. https://doi.org/10.1016/j.brainres.2013.04.042.

Wassum, K. M., Ostlund, S. B., & Maidment, N. T. (2012). Phasic mesolimbic dopamine signaling precedes and predicts performance of a self-initiated action sequence task. *Biological Psychiatry*, *71*, 846–854. https://doi.org/10.1016/j.biopsych.2011.12.019

Weihe, E., Depboylu, C., Schütz, B., Schäfer, M. K.-H., & Eiden, L. E. (2006). Three Types of Tyrosine Hydroxylase-Positive CNS Neurons Distinguished by Dopa Decarboxylase and VMAT2 Co- Expression. *Cell Mol Neurobiol.*, *26*(4–6), 659–678. https://doi.org/10.1007/s10571-006-9053-9

Weingarten, H. P. (1983). Conditioned cues elicit feeding in sated rats: A role for learning in meal initiation. *Science.*, *220*(4595), 431–433. https://doi.org/10.1126/science.6836286

Winstanley, C. A., & Floresco, S. B. (2016). Deciphering Decision Making: Variation in Animal Models of Effort- and Uncertainty-Based Choice Reveals Distinct Neural Circuitries Underlying Core Cognitive Processes. *Journal of Neuroscience*, *36*(48), 12069–12079. https://doi.org/10.1523/JNEUROSCI.1713-16.2016

Worden, L. T., Shahriari, M., Farrar, A. M., Sink, K. S., Hockemeyer, J., Müller, C. E., & Salamone, J. D. (2009). The adenosine A2A antagonist MSX-3 reverses the effort-related effects of dopamine blockade: Differential interaction with D1 and D2 family antagonists. *Psychopharmacology*, *203*(3), 489–499. https://doi.org/10.1007/s00213-008-1396-0

Yamamoto, M., Wada, N., Kitabatake, Y., Watanabe, D., Anzai, M., Yokoyama, M., Teranishi, Y., & Nakanishi, S. (2003). Reversible Suppression of Glutamatergic Neurotransmission of Cerebellar Granule Cells In Vivo by Genetically Manipulated Expression of Tetanus Neurotoxin Light Chain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(17), 6759–6767. https://doi.org/10.1523/JNEUROSCI.23-17-06759.2003

Yohn, S. E., Alberati, D., Correa, M., & Salamone, J. D. (2017). Assessment of a glycine uptake inhibitor in animal models of effort-related choice behavior: implications for motivational dysfunctions. *Psychopharmacology*, *234*(9–10), 1525–1534. https://doi.org/10.1007/s00213-016-4523-3

Yohn, S. E., Errante, E. E., Rosenbloom-Snow, A., Somerville, M., Rowland, M., Tokarski, K., Zafar, N., Correa, M., & Salamone, J. D. (2016). Blockade of uptake for dopamine, but not norepinephrine or 5-HT, increases selection of high effort instrumental activity: Implications for treatment of effort-related motivational symptoms in psychopathology. *Neuropharmacology*, *109*, 270–280. https://doi.org/10.1016/j.neuropharm.2016.06.018

Yohn, S. E., Gogoj, A., Haque, A., Lopez-Cruz, L., Haley, A., Huxley, P., Baskin, P., Correa, M., & Salamone, J. D. (2016). Evaluation of the effort-related motivational effects of the novel dopamine uptake inhibitor PRX-14040. *Pharmacology Biochemistry and Behavior*, *148*, 84–91. https://doi.org/10.1016/j.pbb.2016.06.004

Yohn, S. E., Lopez-Cruz, L., Hutson, P. H., Correa, M., & Salamone, J. D. (2016). Effects of lisdexamfetamine and s-citalopram, alone and in combination, on effort-related choice behavior in the rat. *Psychopharmacology*, *233*(6), 949–960. https://doi.org/10.1007/s00213-015-4176-7

Yohn, S. E., Reynolds, S., Tripodi, G., Correa, M., & Salamone, J. D. (2018). The monoamine-oxidase B inhibitor deprenyl increases selection of high-effort activity in rats tested on a progressive ratio/chow feeding choice procedure: Implications for treating motivational dysfunctions. *Behavioural Brain Research*, *342*, 27–34. https://doi.org/10.1016/j.bbr.2017.12.039

Zarow, C., Lyness, S. A., Mortimer, J. A., & Chui, H. C. (2003) Neuronal Loss Is Greater in the Locus Coeruleus Than Nucleus Basalis and Substantia Nigra in Alzheimer and Parkinson Diseases. *Arch Neurol*, *60*(3), 337–341. https://doi.org/10.1001/archneur.60.3.337

Zhou, Q. Y., & Palmiter, R. D. (1995). Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. *Cell*, *83*(7), 1197–1209. https://doi.org/10.1016/0092-8674(95)90145-0

Zhou, Q. Y., Quaife, C. J., & Palmiter, R. D. (1995). Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature*, *374*(6523), 640–643. https://doi.org/10.1038/374640a0

Zimmerman, C. A., Leib, D. E., & Knight, Z. A. (2017). Neural circuits underlying

thirst and fluid homeostasis. *Nature Reviews Neuroscience*, *18*(8), 459–469. https://doi.org/10.1038/nrn.2017.71

Zimmerman, C. A., Lin, Y. C., Leib, D. E., Guo, L., Huey, E. L., Daly, G. E., Chen, Y., & Knight, Z. A. (2016). Thirst neurons anticipate the homeostatic consequences of eating and drinking. *Nature*, *537*(7622), 680–684. https://doi.org/10.1038/nature18950

Acknowledgement

To everyone I have ever known: You are the very reason why I am here today.

I owe it all to you. Many Thanks!

My utmost appreciation goes to my parents Kao, Chuang-Huei and Kuo, Shwu-Ing, who always supported me unconditionally in my life. I am grateful to my other family members and friends who have inspired me along the way.

I would like to express my sincere gratitude to my advisor Prof. Hisatune for the continuous support of my Ph.D. study, and the labmates Dr. Kaneko, Naohiro Jomura, Teppei Shintani, Junya Koga, Kota Enomoto, and Shota Ando, who helped so much and showed me the living in Japan.

A special gratitude also goes to Prof. Cheng, T.-H. of National Yang-Ming University and Prof. Kojima, Prof. Yamamoto, and Prof. Ugaki of The University of Tokyo for their support and encouragement.

My sincere thanks go to the Japan-Taiwan Exchange Association for generously supporting my Ph.D. scholarship, and the JSPS for funding the work.

Last but not the least, I appreciate Prof. Bolam, Prof. Schiffmann, Prof. D'Aquila, Dr. Trifilieff, and all the anonymous reviewers for providing helpful comments on my article published in European Journal of Neuroscience despite their busy schedules.

Thanks for all your encouragement!