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MICRO REPORT

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Reward-enhancing effect of methylphenidate is abolished in dopamine transporter knockout mice: A model of attention-deficit/hyperactivity disorder

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Abstract

Aim: Attention-deficit/hyperactivity disorder is a heterogeneous neurobiological disorder that is characterized by inattention, impulsivity, and an increase in motor activity. Although methylphenidate has been used as a medication for decades, unknown is whether methylphenidate treatment can cause drug dependence in patients with attention-deficit/hyperactivity disorder. This study investigated the reward-enhancing effects of methylphenidate using intracranial self-stimulation in an animal model of attention-deficit/hyperactivity disorder, dopamine transporter knockout mice.

Methods: For the intracranial self-stimulation procedures, the mice were trained to nosepoke to receive direct electrical stimulation via an electrode that was implanted in the lateral hypothalamus. After the acquisition of nosepoke responding for intracranial self-stimulation, the effects of methylphenidate on intracranial self-stimulation were investigated.

Results: In the progressive-ratio procedure, dopamine transporter knockout mice exhibited an increase in intracranial self-stimulation compared with wild-type mice. Treatment with 5 and 10 mg/kg methylphenidate increased intracranial self-stimulation responding in wild-type mice. Methylphenidate at the same doses did not affect intracranial self-stimulation responding in dopamine transporter knockout mice. We then investigated the effects of high-dose methylphenidate (60 mg/kg) in a rate-frequency procedure. High-dose methylphenidate significantly decreased intracranial self-stimulation responding in both wild-type and dopamine transporter knockout mice.

Conclusions: These results suggest that low-dose methylphenidate alters the reward system (ie, increases intracranial self-stimulation responding) in wild-type mice via dopamine transporter inhibition, whereas dopamine transporter knockout mice do not exhibit such alterations. High-dose methylphenidate appears to suppress intracranial self-stimulation responding not through dopamine transporter inhibition but rather through other mechanisms. These results support the possibility

that methylphenidate treatment for attention-deficit/hyperactivity disorder does not increase the risk of drug dependence, in attention-deficit/hyperactivity disorder patients with dopamine transporter dysfunction.

KEYWORDS
attention-deficit/hyperactivity disorder, dopamine transporter, intracranial self-stimulation, methylphenidate, reward

1 | INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a heterogeneous neurobiological disorder that is characterized by inattention, impulsivity, and an increase in motor activity. The genes that are involved in mesocortical dopaminergic neurotransmission are also considered to be involved in the genetic predisposition to ADHD,[1] including the *dopamine transporter* (*DAT*) gene.[2] Methylphenidate (MPH) is the predominant pharmacotherapy for ADHD. Methylphenidate is a psychostimulant that inhibits both the DAT and norepinephrine transporter (NET).[3] Although MPH has been used as a medication for ADHD for decades, the abuse liability of MPH in patients with ADHD remains unclear.

Dopamine transporter knockout (DAT-KO) mice are one of several transgenic animal models of ADHD that have been developed, based on the postulated role of dopamine in this disorder. Methylphenidate reduces hyperactivity and improves learning in both DAT-KO mice and patients with ADHD. DAT-KO mice have thus been shown to have both face and predictive validities as a model of ADHD.[4,5] Spontaneously hypertensive rats are another model of ADHD, which exhibit a decrease in the reinforcing effects of MPH and a decrease in MPH-seeking behavior.[6] In contrast, the rewarding effects of MPH are less clear in DAT-KO mice. Thus, this study investigated the reward-enhancing effects of MPH using intracranial self-stimulation (ICSS) procedures in DAT-KO mice.

2 | MATERIALS AND METHODS

2.1 | Animals

Heterozygote-heterozygote matings of DAT-KO mice on a C57BL/6J background were used to produce wild-type and homozygous DAT-KO animals.[7] A maximum of 5 mice were housed per cage (one mouse per cage after surgery) in an environment at 23°C ± 1°C with 50% ± 5% relative humidity under a 12-hour/12-hour light/dark cycle. Food and water were available ad libitum. Male and female mice (>20 weeks of age) were used for the experiments, which were conducted under the light phase of the light/dark cycle.

2.2 | Drugs

Methylphenidate and atomoxetine (ATX) were purchased from Sigma (St. Louis, MO, USA) and LKT Laboratories (St. Paul, MN, USA), respectively. Methylphenidate and ATX were dissolved in saline and administered intraperitoneally (i.p.) in a volume of 10 mL/kg body weight 5 min before the experiments.

2.3 | Surgery

A bipolar electrode (BioResearch Center Co. Ltd., Nagoya, Japan) was constructed of 2 tightly twisted strands of insulated stainless steel wire and implanted in the right lateral hypothalamus (0.9 mm posterior, 1.2 mm lateral, 4.9-5.1 mm ventral to bregma) under sodium pentobarbital anesthesia (50 mg/kg, i.p.).

2.4 | Lateral hypothalamic ICSS test procedure

After a 7-day recovery period, the mice were trained to nosepoke for ICSS (0.5 seconds, 100 Hz, 100 μA bipolar square wave) using an ICSS apparatus (Med Associates, St. Albans, VT, USA; see Appendix S1 for details). The mice nosepoked >200 times for ICSS within 10 minutes after 3 training sessions (one training session per day, with >3 days, and between each session). Current amplitude manipulations were then introduced. The current was varied from 50 to 120 μA to determine the optimal current (ie, the weakest current that produced the maximal response rate for each mouse).

Lateral hypothalamic ICSS (lhICSS) responding was investigated after drug treatment using a progressive-ratio schedule[8] or a rate-frequency procedure[9] with the optimal current set for each mouse (see Appendix S1 for details). In the progressive-ratio procedure, the ICSS rate was defined as the percentage of the highest number of nosepokes during the experiment for each mouse. In the rate-frequency procedure, the nosepoke counts for each mouse were normalized to the maximum control rate, which was defined as the maximum rate that was observed at any stimulation frequency during the baseline rate-frequency determination.[9]

2.5 | Statistical analysis

The data are expressed as mean ± SEM. No significant differences were found between male and female mice in the ICSS procedures (see Tables S1 and S2); therefore, we pooled the data for male and female mice. The data were analyzed using two-way repeated-measures analysis of variance (ANOVA) followed by the Sidak multiple comparison post hoc test or Student's *t* test using Prism 7 software

(GraphPad, La Jolla, CA, USA). Values of $P < .05$ were considered statistically significant.

3 | RESULTS

We assessed the reward-enhancing effects of MPH in DAT-KO and wild-type mice using the IhICSS procedure. In the progressive-ratio procedure, naive DAT-KO mice had a significantly higher maximum number of nosepokes in a session compared with naive wild-type mice (wild type: 135.0 ± 28.2; DAT-KO: 302.9 ± 51.7; Student's *t* test, $t = 2.73$, $df = 13$, $P < .05$). Similar to our previous study,[10] DAT-KO mice maintained a high level of ICSS responding and did not reach a progressive-ratio breakpoint. Thus, to evaluate the effects of MPH, we analyzed changes in the ICSS rate-ratio curve using two-way ANOVA (Figure 1). In wild-type mice, significant main effects of ratio and MPH treatment were found, with a significant ratio × MPH treatment interaction (two-way repeated-measures ANOVA; MPH treatment: $F_{3,21} = 5.70$, $P < .01$; ratio: $F_{6,126} = 27.8$, $P < .001$; MPH treatment × ratio interaction: $F_{18,126} = 2.74$, $P < .001$; Figure 1a). The Sidak multiple comparison post hoc test showed that 5 and 10 mg/kg MPH significantly increased the rate of ICSS at ratios of 8, 16, and 32. In DAT-KO mice, MPH treatment at doses of 5-20 mg/kg did not affect the ICSS rate (two-way repeated-measures ANOVA; MPH treatment: $F_{3,30} = 1.80$, $P = .31$; ratio: $F_{6,180} = 6.97$, $P < .001$; MPH treatment × ratio interaction: $F_{18,180} = 0.60$, $P = .90$; Figure 1b). These results showed that MPH at doses of 5-20 mg/kg facilitated reward-related behavior in wild-type mice but not in DAT-KO mice.

Although 20 mg/kg MPH did not significantly affect the ICSS rate, with the exception of ratio 1 in wild-type mice, this dose tended to slightly suppress the ICSS rate in both genotypes of mice. Thus to evaluate the higher dose beyond the therapeutic range of MPH and the selective NET inhibitor ATX, rate-frequency procedures were also performed. In wild-type mice, significant main effects of frequency and drug treatment were found, with a significant frequency × drug treatment interaction (two-way repeated-measures ANOVA; drug treatment: $F_{3,52} = 2.91$, $P < .05$; frequency: $F_{6,312} = 7.76$, $P < .001$; drug treatment × frequency interaction: $F_{18,312} = 4.01$, $P < .001$; Figure 2a). The Sidak multiple comparison *post hoc* test showed that the highest dose of MPH (60 mg/kg) significantly decreased the ICSS rate at frequencies of

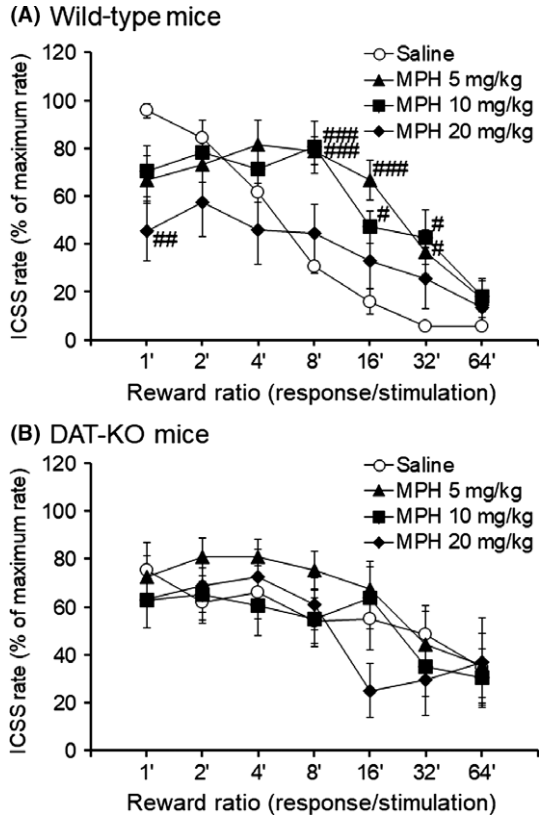

FIGURE 1 Effects of MPH on ICSS rate in the progressive-ratio procedure in (A) wild-type mice (saline, $n = 7$; 5 mg/kg MPH, $n = 6$; 10 mg/kg MPH, $n = 6$; 20 mg/kg MPH, $n = 6$) and (B) DAT-KO mice (saline, $n = 8$; 5 mg/kg MPH, $n = 9$; 10 mg/kg MPH, $n = 9$; 20 mg/kg MPH, $n = 8$). Each mark and each vertical line represent the mean ± SEM. $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, compared with saline-treated group

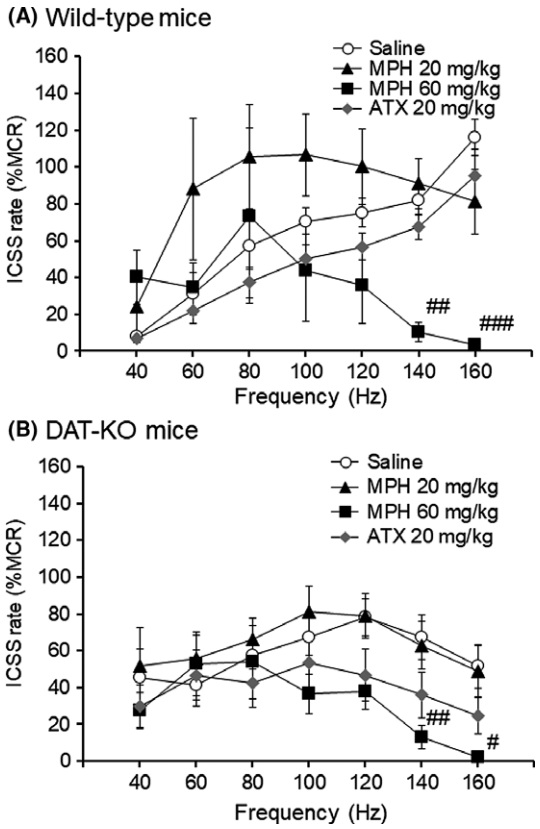

FIGURE 2 Effects of MPH and ATX on ICSS rate in the rate-frequency procedure in (A) wild-type mice (saline, $n = 16$; 20 mg/kg MPH, $n = 8$; 60 mg/kg MPH, $n = 16$; 20 mg/kg ATX, $n = 16$) and (B) DAT-KO mice (saline, $n = 16$; 20 mg/kg MPH, $n = 14$; 60 mg/kg MPH, $n = 16$; 20 mg/kg ATX, $n = 16$). Each mark and each vertical line represent the mean ± SEM. $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, compared with saline-treated group

140 and 160 Hz in wild-type mice. Treatment with 20 mg/kg ATX did not significantly affect the ICSS rate in wild-type mice. Interestingly, in DAT-KO mice, significant main effects of frequency and drug treatment were also found (two-way repeated-measures ANOVA; drug treatment: $F_{3,58} = 3.78$, $P < .05$; frequency: $F_{6,348} = 4.01$, $P < .001$; drug treatment × frequency interaction: $F_{18,348} = 1.03$, $P = .42$). The Sidak multiple comparison post hoc test also showed that 60 mg/kg MPH significantly decreased the ICSS rate at frequencies of 140 and 160 Hz in DAT-KO mice. Furthermore, treatment with 20 mg/kg ATX tended to decrease the ICSS rate in DAT-KO mice, although the decrease was not significant ($P > .1$ at each frequency). Although enhancing noradrenergic neurotransmission has been suggested to facilitate the addiction-related effects of psychostimulants[11], ATX was previously shown to attenuate the effects of dextroamphetamine in humans.[12] Thus, the reward-suppressing effect of high-dose MPH in the present study might be attributable to the strong inhibition of both DATs and NETs.

4 | CONCLUSION

We investigated the reward-enhancing effects of MPH, a medication that is widely used for the treatment of ADHD, in wild-type and DAT-KO mice. These results suggest that low-dose MPH alters the reward system (ie, increases ICSS responding) in wild-type mice via DAT inhibition, whereas DAT-KO mice do not exhibit such alterations. High-dose MPH appeared to suppress ICSS responding not through DAT inhibition but rather through other mechanisms. Noradrenergic neurotransmission might partially regulate the effect of high-dose MPH in DAT-KO mice. These results support the possibility that MPH treatment for ADHD does not increase the risk of drug dependence, at least in a portion of ADHD patients who have some form of DAT dysfunction.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this article.

DATA REPOSITORY

NPPR-2018-014 Tables S1 and S2.xlsx.

AUTHORS CONTRIBUTIONS

SI, YT, and KI involved in the conception and design of the experiments. SI, YI, JH, and YT performed the experiments and statistical analyses and wrote the manuscript. IS, GRU, and KI finalized the manuscript. All of the authors read and approved the final manuscript.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEWER BOARD

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INFORMED CONSENT

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REGISTRY AND THE REGISTRATION NO. OF THE STUDY/TRIAL

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ANIMAL STUDIES

All of the experiments were performed with approval from the Animal Use and Care Committee of the Tokyo Metropolitan Institute of Medical Science.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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