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Research report

Examination of the effects of SCH23390 and raclopride infused in the dorsal striatum on amphetamine-induced timing impulsivity measured on a differential reinforcement of low-rate responding (DRL) task in rats

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ABSTRACT

Although the striatal dopamine (DA) is reportedly involved in impulsive action, little is known about the DA subtype receptors of dorsal striatum (dSTR) in the impulsive control involved in differential reinforcement of low-rate-responding (DRL) behavior. We examined the receptor-specific dopaminergic modulation of d-amphetamine (AMP)-altered DRL 10 s (DRL-10 s) performance by locally infusing SCH23390 (SCH) and raclopride (RAC), DA D1 and D2 receptor antagonists, respectively, into the rat's dSTR. Systemic injection of AMP significantly affected DRL-10 s behavior by increasing total, non-reinforced, and bust responses, as well as by decreasing reinforced responses, which correspondingly caused a leftward shift of the inter-response-time distribution curve as confirmed by a profound decrease in peak time (i.e., < 10 s). Neither SCH nor RAC into dSTR pharmacologically reversed the timing impulsivity produced by AMP as measured by non-reinforced responses and peak time. However, the increase in total responses and the decrease in reinforced responses by AMP were reversed by intra-dSTR SCH or RAC. These results suggest that the D1 and D2 receptors of the dSTR may be involved in behavioral components apart from the timing impulsivity produced by AMP on a DRL task, which components are distinctly different from those in other terminal areas of midbrain DA systems.

1. Introduction

Brain dopamine (DA) is known to be important for modulating impulsive control [1]. However, the neural mechanisms underlying impulsivity remain elusive. Several rat behavioral models with specific constructs of impulsivity have been developed to study the neuropsychopharmacology of impulsive behavior [2]. Impulsive action, as one of the two key facets of impulsivity, is defined as the failure to withhold a response and thus manifest poor response inhibition. The 5-choice serial reaction time (5-CSRT) task and differential reinforcement of low-rate responding (DRL) schedule-controlled behavior are rodent models used for assessing impulsive action related to the inhibitory dysfunction of “failing to wait.” Notably, most research findings have been derived from the studies using 5-CSRT task, but not DRL behavior [3]. Behavioral components involved in these two tasks are thought to be different. Unlike the 5-CSRT, the DRL behavior does not involve attentional engagement to external visual cue/signal. Instead, a more implicit cognitive process of "wait" in time is required for optimal response in the DRL task. Thus, we postulated that neural substrates and pharmacological mechanisms underlying these two behaviors of impulsive action could be different.

A considerable number of studies have shown that DRL operant response is affected by psychostimulant drugs [4]. In an attempt to decipher its neural mechanisms, we recently reported an increase in non-reinforced (or premature) responses and a decrease in peak time as produced by the systemic administration of d-amphetamine (AMP), which may represent a timing-dysregulated impulsive action [5]. A regional-dependent and DA receptor subtype specific effect across the medial prefrontal cortex (mPFC), the nucleus accumbens (NAC; known as the ventral striatum), and the dorsal hippocampus (dHIP) has been found for this timing impulsivity induced by AMP on DRL behavior. Although these findings support the involvement of corticostriatal circuits in impulsive action or behavioral inhibition [6–8], the potential roles in modulating the aforementioned timing impulsivity in the dorsal

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striatum (dSTR) remain unexplored. Despite that striatal subareas collectively share some anatomical and neurochemical similarities, dSTR and NAC are functionally distinct in terms of reward motivation and cognitive control [9–12]. Accumulating evidence suggests the presence of dopaminergic mechanisms in the dSTR underlying the interval timing within the range of seconds [13,14] and inhibitory control of motor impulsivity [8,15]. On the basis of these findings, the present study sought to investigate the role of DA subtype receptors of the dSTR in timing impulsivity. By locally infusing SCH23390 (a D1 receptor antagonist) and raclopride (a D2 receptor antagonist) in dSTR, we evaluated the effects of D1- or D2-receptor blockade in the dSTR on AMP-altered DRL behavior.

2. Materials and methods

2.1. Subjects

Twenty male Wistar rats, averaged approximately 250 g in body weight, were obtained from the Center of Experimental Animals, National Taiwan University Hospital. The rats were provided with food and water provided *ad libitum* and housed in a colony with the vivarium's 12 h/12 h light/dark cycle (light on at 07:30). The temperatures of the colony and of the behavioral test room were maintained at 23 ± 1 °C throughout the experiments. After adaptation for 10 days with the experimenter's daily handling, the rats received a water-restriction regimen such that there was 10 ± 5 min access to tap water in the home cage occurring no sooner than 30 min after the end of each daily experimental session. The body weight was carefully monitored and allowed to increase throughout the entire experiment on a delayed-growth curve. All procedures were approved by an institutional review committee.

2.2. Apparatus

Behavioral trials were conducted using four operant chambers (MED Associated), which were served by a microcomputer with an in-house designed program to control the operant environment and data collection [5]. The interior dimensions of each chamber were 20 cm x 25 cm x 30 cm (MED Associated, St. Albans, VT, USA). Aluminum panels formed the front and back walls, and clear Plexiglas comprised the remaining sides and the top. Stainless-steel rods (diameter = 5 mm) were set 11 mm apart to provide flooring. Each chamber was equipped with a lever positioned 7.3 cm above the floor and 4 cm from the right corner of the front panel. A liquid dispenser was set outside of the front panel of the chamber. The reinforcer delivery mechanism provided 0.04 ml of tap water at each correct/reinforced response. The water was delivered into a receiving dish (diameter = 25 mm) located at the center of the front panel and at 2 cm above the floor. The chamber was illuminated by a small light bulb located 10 cm above the floor and positioned 5 cm from the left corner of the front panel. Each chamber was enclosed in a plywood box with a fan for ventilation and to mask any outside noise.

2.3. Surgery

A standard stereotaxic operation for the bilateral implantation of stainless-steel cannulae was conducted under sodium pentobarbital anesthesia (40 mg/kg; IP). The coordinates for the final injection site of the dSTR were as follows: AP = +0.7 mm from bregma, L = ± 2.5 mm, D = -5.5 mm relative to the dura [16]. The location of dSTR was chosen according to a previous study [17]. At the end of surgery, penicillin (50,000 I.U.) was administered intramuscularly to reduce the likelihood of post-operative infection. Subjects were allowed 7–9 days to recover from surgery before the behavioral test with pharmacological manipulations.

2.4. Drugs and microinjection

D-amphetamine sulfate (Sigma), SCH23390 hydrochloride (Tocris Cookson), and raclopride L-tartrate (RBI) were dissolved in 0.9 % physiological saline. Microinjection of SCH or RAC into dSTR was done by 28-gauge injection needle connected by PE20 tubing to 2 μl Hamilton micro-syringe. Each drug or vehicle solution was locally infused in 0.25 μl over 1 min per site. The injector needle was extended from the bottom of the guide cannulae for 1.5 mm. After infusing the drug or vehicle, the needle was left in place for one more minute to allow diffusion from the injection site and to reduce the possibility of reflux. The low and high doses were 1.5 and 5 nmol for SCH, and those of 1.5 and 15 nmol were for RAC. The selection of intra-dSTR injection doses of SCH and RAC was based on previous studies [18,19].

2.5. Procedures

The subjects received operant behavior trainings after adaptation to the water- restriction regimen. They were initially trained to press the lever to obtain water as a reinforcer under fixed-ratio 1 (FR 1) schedule for seven sessions before entering the DRL training. In the DRL task, the rats had to wait a specified number of seconds between lever presses in order to obtain the reinforcer. Any response made before the criterion time would reset the DRL clock. The subjects were trained with the schedule of DRL-5 s for approximately 15 daily sessions. Subsequently, the DRL criterion time was increased from 5 to 10 s, in which a lever response made in 10 s or more after the prior response was reinforced by water. The DRL-10 s training phase lasted for 30 daily sessions before the intra-dSTR cannulation surgery was performed. After post-surgery recovery, the rats were run for five sessions on the same DRL-10 s task to ensure a stable baseline before drug tests. The behavioral session of training or testing was 15 min each day.

For pharmacological testing, 10 rats were assigned to the SCH treatment group, whereas another 10 to the RAC treatment group. The intraperitoneal (i.p.) injection of AMP or saline vehicle (SAL) was done 15 min before the behavioral session commenced; microinjection of SCH, RAC, or vehicle was conducted immediately before AMP injection. The dose of AMP (1 mg/kg) was selected based on previous reports [4,5], specifically avoiding the unwanted operant-response interference. In both SCH and RAC group, each rat underwent six pharmacological tests. On each of the six drug test days, the subject received two drug administrations, one being systemic and the other one being intra-dSTR microinjection. The pharmacological tests were conducted in the following order: a saline microinjection with a saline i.p. injection (SAL-SAL); a saline microinjection and an AMP i.p. injection (SAL-AMP); a microinjection of DA receptor antagonist at low dose with a saline i.p. injection (SCH-SAL or RAC-SAL); a microinjection of low-dose DA receptor antagonist with AMP i.p. injection (SCH-AMP or RAC-AMP); a microinjection of DA receptor antagonist at high dose with a saline i.p. injection; and a microinjection of high-dose DA receptor antagonist with AMP i.p. injection. Conducting the first two drug tests (i.e., SAL-SAL and SAL-AMP) allowed us to verify the significance of AMP treatment alone before entering the drug-combined tests of AMP and DA receptor antagonist. In the last four tests, the high and low doses of DA antagonist given alone or with AMP were counterbalanced across the subjects within the group. Between each of these drug tests, at least one daily session of DRL training was conducted to ensure a stable baseline and wash out drug carry-over effect.

2.6. Histology

After the completion of drug tests, the subjects received an overdose of sodium pentobarbital and were then perfused intracardially with saline followed by 10 % formalin. Following fixation, the brain was sectioned at 40 μm with a freezing microtome. The mounted slices were stained with cresyl violet to verify the locations of microinjection.

2.7. Data collection and statistics

Each lever press was classified in terms of its associated inter-response-time (IRT; the time in millisecond elapsed since the prior response), and the resulting dataset on IRT was grouped and plotted into a distribution consisting of response frequencies for 21 consecutive 1 s time bins. For quantitative analyses, six dependent variables were studied: 1) total responses; 2) reinforced responses, lever press with IRT > = 10 s; 3) non-reinforced responses, lever press with IRT $<$ 10 s; 4) burst responses, lever response with IRT $<$ 2 s; 5) peak rate; and 6) peak time. The peak time and peak rate were calculated from the de-burst IRTs (IRT $>$ 2 s), in which a moving average based on four consecutive 1 s bins with a 1 s step size was applied to smoothen the distribution. After identifying the maximum frequencies for a 4 s epoch, the peak time was designated as the average value (in millisecond) of all IRTs that fell within the four bins (i.e., the maximal epoch). The peak time measurement indicated at which time point the rats pressed the lever with the highest IRT frequency, i.e., their expected time for obtaining the reinforcer. The peak rate was calculated from the summed responses in the aforementioned four bins divided by four. It indicated how strongly the rats were motivated to press the lever at the expected criterion time. This smoothing procedure has been previously used [5].

A few cases of missing data were observed due to a high level of balking under drug treatment. The subjects had only made 15 lever presses or less within a session. For each case, during statistical analysis, the missing data were replaced by the group mean of available cases in the same group receiving the same drug treatment (mean imputation) [20]. Furthermore, stochastic regression imputation, which provides the most likely value of missing data by regression of the available cases, was implemented for comparison with the aforementioned mean imputation method. The analysis was done by using open-source R software with the available package "Mice" [21,22].

Each of the six measures was separately subjected to one-way repeated measures ANOVA and followed by a Fisher LSD *post hoc* test if the ANOVA test was significant [23]. The significance level was set at $p < 0.05$ for all tests. The data were all presented as means ± S.E.M.

3. Results

Only 16 rats (n = 8 for each group) that had bilateral needle tracks terminating in dSTR were included in data analysis (Fig. 1). In either SCH or RAC group, two subjects among the initial 10 rats were excluded because one did not have symmetrical intra-dSTR cannula implantation and the other one had its cannulation come off the head before the completion of pharmacological tests.

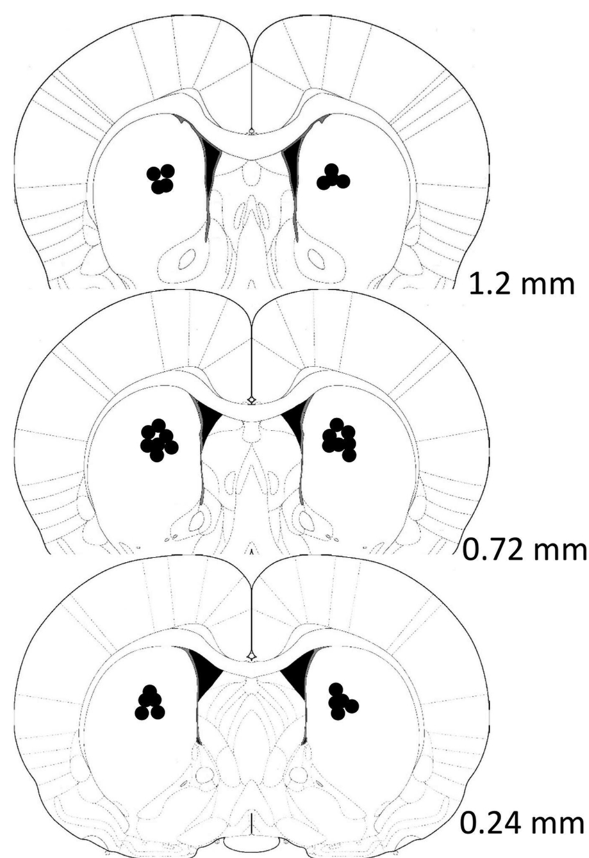

Fig. 1. Distribution of infusion needle tips in the dorsal striatum obtained from the experimental subjects of two groups. The plates of coronal brain sections were adapted from Paxinos and Watson [16].

The effects of AMP alone and in combination with intra-dSTR infusion of SCH or RAC are shown in Figs. 2 and 3, respectively. In terms of IRT distributions from the groups that received SCH (Fig. 2A) and RAC (Fig. 3A), remarkable typical IRT curves were obtained from the DRL procedure depicting bi-modal distributions. The first mode was around the very short IRT bins (< = 2 s; as burst response), and the second mode was around the criterion time (i.e., 10 s). These modes were observed under the control conditions (SAL-SAL) in both SCH and RAC groups. Systemic injection of AMP (SAL-AMP) shifted the IRT curve to the left. This left-ward shift of the IRT curve corresponded to the decrease of reinforced responses and the increase of non-reinforced responses.

For the non-reinforced response, the ANOVA yielded significant differences in both SCH-treated ($F_{(5,35)} = 11.9, p < 0.001$; Fig. 2B) and RAC-treated ($F_{(5,35)} = 4.8$, $p < 0.01$; Fig. 3B) rats. *Post hoc* comparisons revealed that the differences were observed under the following conditions in the SCH group: 1) SAL-AMP ($p < 0.001$), 2) 1.5 nmol SCH-AMP ($p < 0.001$), and 3) 5.0 nmol SCH-AMP ($p < 0.05$), all compared with the control condition of SAL-SAL (Fig. 2B). Thus, both doses of SCH did not decrease the non-reinforced response that was increased by systemic AMP injection to a level comparable with that of the control condition, although a decreasing trend was observed for the high dose (5 nmol SCH-AMP; $p = 0.043$). In terms of RAC, results of *post hoc* tests indicated that both RAC doses failed to decrease the non-reinforced response that was increased by the systemic AMP injection, as shown in Fig. 3B ($p < 0.01$ for 1.5 nmol RAC-AMP and $p < 0.05$ for 15 nmol RAC-AMP).

In peak time, significant treatment effects appeared in both SCH-treated and RAC-treated groups, $F_{(5,35)} = 12.5$, $p < 0.001$ (Fig. 2C) and $F_{(5,35)} = 5.0$, $p < 0.01$ (Fig. 3C), respectively. *Post hoc* comparisons revealed that SCH failed to reverse the effect of systemic AMP on peak time because a significant difference existed between the SAL-SAL and under each of the following three conditions: 1) SAL-AMP, 2) 1.5 nmol SCH-AMP and 3) 5.0 nmol SCH-AMP (all $p < 0.001$, Fig. 2C). For the RAC group (Fig. 3C), compared with its own SAL-SAL condition, significant differences were detected in AMP treatment alone and both doses of RAC in conjunction with systemic AMP injection (all $p < 0.01$). The RAC also failed to reverse the peak time altered by systemic AMP injection.

To further examine the potential effects of antagonist drugs on reversing the effects of systemic AMP injection, we performed a repeated-measure two-way ANOVA (2 × 3) with two doses of AMP and three doses of antagonist. In terms of non-reinforced response, no interaction effect was observed on either SCH ($F_{(1,2)} = 0.92$, $p = 0.42$) or RAC ($F_{(1,2)} = 0.26$, $p = 0.773$). No interaction effect was observed for the measure of peak time for both drugs ($F_{(1,2)} = 0.39$, $p = 0.685$ and $F_{(1,2)} = 0.58$, $p = 0.571$ for SCH and RAC, respectively). Hence, neither SCH nor RAC was shown to interact with AMP at given doses on these two measures.

Furthermore, to detect any potential difference between SCH and

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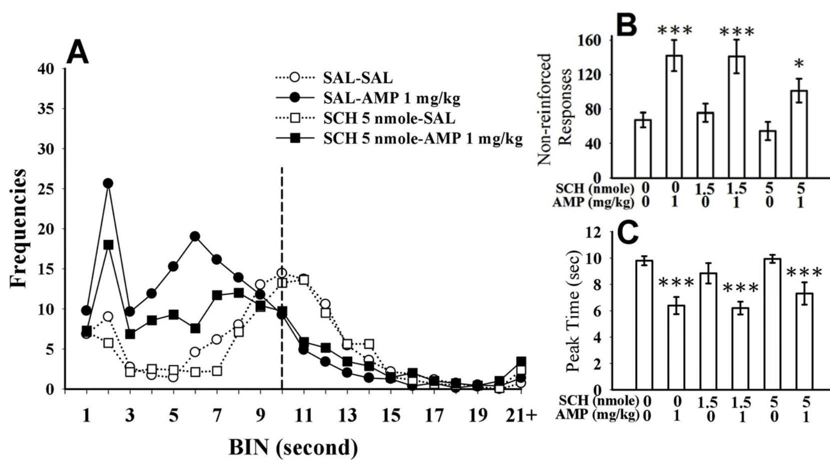

Fig. 2. Effects of SCH23390 (SCH) locally infused in the dorsal striatum on d-amphetamine (AMP) induced behavioral changes in the DRL-10 s task. (A): IRT distributions from the group of rats ($n = 8$) tested for SCH under treatments with the saline control (SAL-SAL; open circles), AMP treatment alone (SAL-AMP; filled circles), SCH alone (SCH-SAL; open squares), and combined treatment of SCH and AMP (SCH-AMP; filled squares). The two IRT distribution curves regarding the treatment with a low dose of SCH with saline and its combination with AMP are not included in (A) for the clarity of the figure. (B): Non-reinforced responses of DRL behavior under the treatments are denoted. (C): Peak times derived from IRT data under the treatments are denoted. * $p < 0.05$, *** $p < 0.001$, compared with SAL-SAL treatment.

RAC, a mixed-effect of two-way ANOVA was conducted with SCH and RAC as the between-subjects factor (two levels) and all drug treatments as repeated measures (six levels). In results, there was no interaction effect between the two factors on non-reinforced response ($F_{(5,70)} = 1.05$, $p = 0.397$) and peak time ($F_{(5,70)} = 0.72$, $p = 0.611$). With regard to the main effect of the two drugs, no significant difference was observed for the non-reinforced response ($F_{(1,14)} = 0.07$, $p = 0.796$) and the peak time ($F_{(1,14)} = 0.07$, $p = 0.794$). Given the aforementioned within-subject factor can be further separated into AMP dose (2-level) and DA antagonist dose (3-level), a grand 3-way ANOVA (2 × 2 × 3) was conducted in this manner. The results did not confirm any significant interaction in both measures of non-reinforced responses ($p = 0.84$ for 3-way interaction) and peak time ($p = 0.68$ for 3-way interaction). Only a significant main effect of AMP was observed in the results ($p < 0.001$ and $p < 0.001$, respectively). Therefore, there was no difference between SCH and RAC in their ability to alter the DRL performance at given doses with or without AMP.

The results of the other four measures in these two groups are shown in Table 1. For the SCH group, ANOVA yielded significant differences in drug treatment effects on total responses ($F_{(5,35)} = 5.91$, $p < 0.001$), reinforced responses ($F_{(5,35)} = 12.15$, $p < 0.001$), burst responses ($F_{(5,35)} = 2.67$, $p < 0.05$), and peak rate ($F_{(5,35)} = 2.72$, $p < 0.05$). For the RAC group, significant drug treatment effects were detected on the total responses ($F_{(5,35)} = 3.32$, $p < 0.05$), and reinforced responses ($F_{(5,35)} = 9.92$, $p < 0.001$), but not on burst responses ($F_{(5,35)} = 1.43$, $p = 0.24$) and peak rate ($F_{(5,35)} = 2.17$, $p = 0.08$). As revealed by *post hoc* tests, the increase in total responses and the decrease in reinforced responses were reversed by intra-dSTR SCH or RAC only at high dose. The AMP-increased burst responses were reversed only at the high SCH dose. The AMP-increased peak rate was reversed by both doses of SCH. Although the trends of reversing AMP-induced increase in burst responses and peak rate appeared in the RAC group, neither had the support of ANOVA main effects.

Finally, to address the concern of underestimating group variance of mean imputation method in replacing the missing data, we conducted stochastic regression imputation, which is a more robust imputation method, on the current data set. As shown in Table 2, the results are identical between these two imputation methods. Therefore, our conclusions based on the mean imputation methods are still established.

4. Discussion

We demonstrated timing impulsivity produced by AMP on a DRL-10 s task as specifically determined by the increase in non-reinforced responses and the decrease in peak time. AMP treatment alone also significantly increased the total responses and decreased the reinforced responses. Consistent with our previous results [e.g., 5], AMP alone significantly altered DRL-10 s behavior by producing a leftward shift of the IRT response curve, as confirmed by a remarkable decrease in peak time along with increasing non-reinforced responses. These results showed an impulsive action with a faster internal clock speed for the timing as produced by AMP. The present quantitative analyses of IRT distribution provide a unique means to verify AMP-induced timing impulsivity.

Surprisingly, the increase in non-reinforced responses and decrease in peak time by AMP were not significantly reversed by either SCH or RAC infused into the dSTR. This result indicated that neither D1 nor D2 receptors alone in the dSTR were required in the modulation of the

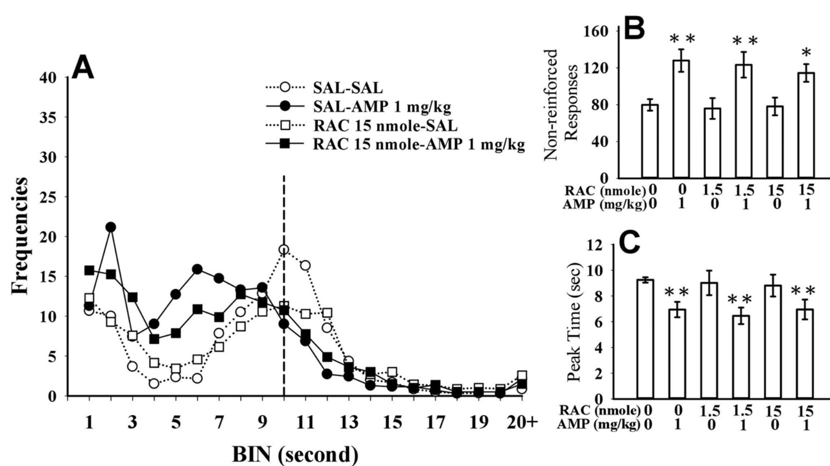

Fig. 3. Effects of raclopride (RAC) locally infused in the dorsal striatum on d-amphetamine (AMP) induced behavioral changes in the DRL-10 s task. (A): IRT distributions from the group of rats ($n = 8$) tested for RAC under treatments with the saline control (SAL-SAL; open circles), AMP treatment alone (SAL-AMP; filled circles), RAC alone (RAC-SAL; open squares), and combined treatment of RAC and AMP (RAC-AMP; filled squares). The two IRT distribution curves regarding the treatment with a low dose of RAC with saline and its combination with AMP are not included in (A) for the clarity of the figure. (B): Non-reinforced responses of DRL behavior under the treatments are denoted. (C): Peak times derived from IRT data under the treatments are denoted. * $p < 0.05$, ** $p < 0.01$, compared with SAL-SAL treatment.

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Table 1
The effects of SCH23390 and raclopride locally infused into the dorsal striatum on d-amphetamine (AMP; 1 mg/kg, i.p.) induced behavioral changes in the DRL-10 s task on four quantitative measures.

	SCH23390 (SCH, n = 8) Low: 1.5 nmol; High: 5 nmol					Raclopride (RAC, n = 8) Low: 1.5 nmol; High: 15 nmol				
	Total Response	Reinforced Response	Burst Response	Peak Rate	No. of missing data	Total Response	Reinforced Response	Burst Response	Peak Rate	No. of missing data
SAL-SAL	106.4 ± 5.6	38.6 ± 4.7	15.9 ± 3.0	14.0 ± 1.3	1	113.8 ± 7.0	34.0 ± 3.0	20.7 ± 7.7	15.1 ± 0.6	2
SAL-AMP	*158.0 ± 13.	*15.9 ± 4.4	*35.4 ± 6.8	*18.1 ± 2.0	0	*146.6 ± 11.	*18.6 ± 3.7	32.4 ± 11.	17.8 ± 2.0	1
Low-SAL	111.6 ± 9.4	35.6 ± 3.4	19.3 ± 5.2	14.3 ± 0.9	1	112.3 ± 7.9	36.4 ± 3.8	19.3 ± 5.6	13.4 ± 1.4	0
Low-AMP	*159.4 ± 19.	*18.1 ± 3.7	*38.9 ± 15.	17.5 ± 2.4	1	*144.1 ± 12.	*20.8 ± 2.1	37.6 ± 11.	16.2 ± 1.5	0
High-SAL	94.3 ± 10.	39.3 ± 3.5	12.9 ± 4.9	12.3 ± 1.4	0	115.0 ± 8.5	36.9 ± 2.7	21.6 ± 7.8	11.8 ± 1.4	1
High-AMP	127.7 ± 13.	26.3 ± 3.3	25.3 ± 7.7	14.0 ± 2.1	1	140.4 ± 9.3	25.9 ± 1.9	31.0 ± 6.2	15.6 ± 1.9	0

Note: Details of four quantitative measures of the DRL-10 s performance are depicted in Data Collection and Statistics (Section 2.7). The data points with significant *post-hoc* test results in comparison with the SAL-SAL condition are denoted with a "*" and underlined. Data are represented as Mean ± SEM.

AMP-produced timing impulsivity. This negative result is in contrast with the previously reported effects of these two DA receptor antagonists infused in NAC, mPFC, and dHIP [5]. Specifically, based on heterogeneous functions of the *striatal* subregions, the effects of DA receptor blockade between dSTR and NAC are worth comparing. Intra-NAC SCH reversed the peak time and the non-reinforced responses altered by AMP, whereas intra-dSTR SCH only partially reversed the non-reinforced responses (Fig. 2B). Even though SCH didn't completely reverse the effects of AMP, the partial reversal by SCH suggests that DS D1 plays at least a minor role in AMP-induced timing impulsivity, although it is clearly not 100 % accountable for AMP's effects. In either NAC or dSTR, RAC did not reverse the peak time and the non-reinforced responses altered by AMP. In general, the involvement of striatal D2 receptors in the AMP timing impulsivity on DRL behavior is minimal, whereas the association of striatal D1 receptors can be regionally dependent. In line with this inference, the current data did not entirely denote the lack of the pharmacological antagonism of SCH and RAC on AMP-altered DRL responses. The intra-dSTR SCH or RAC given at high dose did reverse the increase in total responses and the decrease in reinforced responses by AMP back to the SAL-SAL control level statistically. And, intra-dSTR SCH treatment dose-dependently attenuated the AMP-increased burst responses and peak rate (Table 1, left side). Thus, dSTR DA receptors may be involved in modulating behavioral components other than those related to timing impulsivity under AMP treatment on the DRL task, such as motivation shown in the index of peak rate. The presented data support the notion of heterogonous functions between dorsal and ventral regions of the striatum for impulsive action.

Systemic injection of AMP produces waiting and/or attentional impulsivity by increasing premature responses in 5-CSRT task [24–28]. With the mesocorticolimbic DA systems being noted with the 5-CSRT impulsive action [29], dissociable effects of focal lesions made in dSTR and/or its subareas have been reported. The lesion of dorsolateral part severely impaired behavioral performance, whereas the lesion of dorsomedial area selectively increased premature responses made by the rat during the inter-trial interval [30]. The effect of dorsomedial lesion was also shown in rats with functional disconnection between dSTR and mPFC [31]. Differential effects of intra-dSTR D1 and D2 receptor antagonists on 5-CSRT impulsive action induced by intra-mPFC CPP (an NMDA receptor antagonist) were observed [32]. Intriguingly, DA depletion by 6-hydroxydopamine in the dSTR was shown to affect response vigor with accuracy and the impulsive response of 5-CSRT [33]. However, such a dSTR DA depletion did not reverse AMP-increased premature responses of 5-CSRT, whereas NAC DA depletion attenuated this drug-induced impulsive action [34]. Despite that these findings indicated that striatal subareas are differentially involved in 5-CSRT, no study has yet directly examined whether blockade of D1 and D2 receptors in the dSTR could ameliorate impulsive action induced by the systemic injection of AMP on 5-CSRT. Nonetheless, it is conceivable to infer that 5-CRST impulsive action modulated by D1 and D2 receptors

Table 2
Comparisons of mean imputation and stochastic regression imputation on data from SCH23390 and raclopride treatment groups.

	Mean imputation			Stochastic regression imputation		
	Non-reinforced Response	Peak Time	Missing data	Non-reinforced Response		Peak Time
(A) D1 receptor antagonist group (SCH23390, n = 8) Low: 1.5 nmol; High: 5 nmol						
SAL-SAL	67.9 ± 8.6	9.8 ± 0.3	1	68.5 ± 8.6	9.6 ± 0.4	
SAL-AMP	*142.1 ± 18.1	*6.4 ± 0.6	0	*142.1 ± 18.1	*6.4 ± 0.6	
Low-SAL	76.0 ± 10.4	8.8 ± 0.8	1	82.0 ± 12.0	8.6 ± 0.8	
Low-AMP	*141.3 ± 19.5	*6.2 ± 0.5	1	*147.4 ± 20.4	*6.2 ± 0.5	
High-SAL	55.0 ± 10.5	9.9 ± 0.3	0	55.0 ± 10.5	9.9 ± 0.3	
High-AMP	*101.4 ± 13.7	*7.3 ± 0.7	1	*108.5 ± 15.4	*7.5 ± 0.9	
(B) D2 receptor antagonist group (Raclopride, n = 8) Low: 1.5 nmol; High: 15 nmol						
SAL-SAL	79.8 ± 6.3	9.3 ± 0.2	2	72.0 ± 8.1		9.5 ± 0.3
SAL-AMP	*128.0 ± 12.2	*6.9 ± 0.6	1	*130.6 ± 12.5		*6.7 ± 0.7
Low-SAL	75.9 ± 11.3	9.0 ± 1.0	0	75.9 ± 11.3		9.0 ± 1.0
Low-AMP	*123.4 ± 13.9	*6.5 ± 0.6	0	*123.4 ± 13.9		*6.5 ± 0.6
High-SAL	78.1 ± 9.6	8.8 ± 0.8	1	74.5 ± 10.3		8.6 ± 0.9
High-AMP	*114.5 ± 9.6	*7.0 ± 0.8	0	*114.5 ± 9.6		*7.0 ± 0.8

Note: Two quantitative measures of the DRL-10 s performance – Non-reinforced response and peak time are listed here to illustrate the difference between mean imputation and stochastic regression imputation in replacing the missing data. The two imputation methods yielded identical statistical results, although there is a slight difference between the means and SEMs in the groups with missing data. (A) data from the SCH treatment group; (B) data from the RAC treatment group. The data points with significant Fisher LSD *post-hoc* test results in comparison with the SAL-SAL condition are denoted with a "*" and underlined. Data are represented as Mean ± S.E.M.

in different DA terminal areas may differ from DRL's.

The findings of this study need to be considered in the following limitations. First, the dSTR as the targeted area for the intracranial infusion of DA receptor antagonists was primarily designed to compare with the NAC, which was tested in the same experimental protocols as our previous study [5]. Although comparing the dorsal and ventral regions of the stratum is pertinent, there could be a limitation in regarding to the dSTR location of the present study. A substantial body of evidence suggests that the medial and lateral subareas of dSTR can be further divided, noted as the dorsomedial and dorsolateral parts of striatum (dmSTR and dlSTR) in terms of anatomical and functional heterogeneity [35,36]. Based on previous findings that the posterior (but not anterior) region of dmSTR is critically important in instrumental conditioning [37], this issue may also involve the anterior-posterior axis of the dSTR to dissect the distinct functions of dSTR subareas for future studies. Second, further testing for simultaneous blockade of D1 and D2 receptors may be needed to characterize the role of DA receptor in dSTR on the present task. AMP can massively increase the synaptic level of DA, which could then simultaneously activate D1 and D2 receptors. Given that the synergistic effect of D1 and D2 agonist actions exists in striatal tissue [38], blocking only one receptor subtype at a time may not be sufficient in dSTR to reverse the AMP effect in the present study. Third, the effects of systemic AMP injection on DRL behavioral performance may be due to enhanced DA (and possibly a contribution from norepinephrine or serotonin) transmission in a number of brain areas at once and all together. Although this notion supports the involvement of corticostriatal circuits in impulsive action as measured by DRL task, the interactions between dSTR and each of the other brain sites remain unknown. The functional disconnection approach with the tools of lesion and pharmacological inaction may be used to address this issue. In addition, the use of in-vivo chemogenetic or optogenetic tools may help elucidate the neural mechanisms underlying the impulsive action measured by DRL task that are presumably different from those of 5CSRTT and the other types of impulsivity [39].

In conclusion, this study replicated the results of our previous work, which showed AMP-produced timing impulsivity on a DRL behavioral task. The null results of intra-dSTR SCH and RAC treatments on the reversal of AMP-altered non-reinforced responses and peak time indicated the minimal involvement of dSTR D1 and D2 receptors in timing impulsivity as measured by DRL, at least in the range of doses tested here. The effects observed here add to an existing body of knowledge on the neurobehavioral mechanisms of impulsive action, and may elucidate the distinctive roles of DA receptor subtypes across brain areas in modulating attentional and time-based impulsivity.

Declaration of Competing Interest

The authors declare no conflict of interest.

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