Acute Effects of d-Amphetamine on the Differential Reinforcement of Low-Rate (DRL) Schedule Behavior in the Rat: Comparison with Selective Dopamine Receptor Antagonists

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Abstract

Amphetamine and it analogs have been shown to affect operant behavior maintained on the differential reinforcement of a low-rate (DRL) schedule. The aim of the present study was to investigate what specific component of the DRL response is affected by d-amphetamine. The acute effects of d-amphetamine on a DRL task were compared with those of the selective dopamine D_1 and D_2 receptor antagonists, SCH23390 and raclopride, respectively. Pentylenetetrazole and ketamine were also used as two reference drugs for comparison with d-amphetamine as a psychostimulant. Rats were trained to press a lever for water reinforcement on a DRL 10-s schedule. Acute treatment of d-amphetamine (0, 0.5, and 1.0 mg/kg) significantly increased the response rate and decreased the reinforcement in a dose-related fashion. It also caused a horizontal leftward shift in the inter-response time (IRT) distribution at the doses tested. Such a shifting effect was confirmed by a significant decrease in the peak time, while the mean peak rate and burse response remained unaffected. In contrast, both SCH23390 (0, 0.05, and 0.10 mg/kg) and raclopride (0, 0.2, and 0.4 mg/kg) significantly decreased the total, non-reinforced, and burst responses. The de-burst IRT distributions were flattened out as shown by the dose-related decreases in the mean peak rate for both dopamine antagonists, but no dramatic shift in peak time was detected. Interestingly, neither pentylenetetrazole (0, 5, and 10 mg/kg) nor ketamine (0, 1, and 10 mg/kg) disrupted the DRL behavioral performance. It is then conceivable that d-amphetamine at the doses tested affects the temporal regulation of DRL behavior. The effectiveness of d-amphetamine is derived from its drug action as a psychostimulant. Taken together, these data suggest that different behavioral components of DRL task are differentially sensitive to pharmacological manipulation.

Key Words: D_1 and D_2 receptors, IRT analysis, ketamine, pentylenetetrazole, psychostimulant, raclopride, SCH23390, timing behavior

Introduction

Amphetamine, classified as a psychostimulant drug with high abuse liability, produces a variety of behavioral effects (18, 42). Among many measures, loss of behavioral inhibition and overestimation of the perception of time are the two main effects which can be observed with the use of amphetamine or its derivatives by human subjects (8, 24). In laboratory animals, operant behavior maintained on the differential reinforcement of low-rate responding (DRL) schedule has been characterized with temporal regulation as well as behavioral inhibition. Rats trained on this schedule of reinforcement are required to inhibit or withhold responding for a minimum specified period of time in order to obtain a reinforcer. Acute treatment with amphetamine at moderate doses (0.5~3.0 mg/kg) was demonstrated to disrupt DRL behavioral performance by increasing the number of responses and decreasing the number of reinforcers

obtained (2, 3, 5, 6, 34, 36, 38, 39, 41, 50). In addition to the measures of the aforementioned response and reinforcement rates, the inter-response time (IRT) specified for each response is now recognized as a useful measure to evaluate the drug effects on DRL behavioral performance. However, only a limited number of previous studies have reported the effects of amphetamine on the IRT frequency distribution. Of those few studies, some presented a qualitative analysis of the IRT data in order to address the way that amphetamine alters DRL behavior (28, 36). Amphetamine, in general, causes rats to respond with shorter IRTs leading a leftward shift in the IRT frequency distribution. Although the pharmacological properties remain controversial, it has been suggested that these behavioral changes in the DRL schedule produced by amphetamine are predominately mediated by dopamine released in the brain (38). Thus, it might be expected that a direct comparison of the effects of amphetamine on a specific DRL behavior with those produced by dopamine-related drugs might be of importance for further delineating this issue.

Indeed, disruption of DRL behavioral performance has been demonstrated in the rat undergoing systemic and acute treatment with typical neuroleptic drugs including haloperidol (3, 48, 49), pimozide (27), and chlorpromazine (5, 40). Although these neuroleptic drugs consistently produce a dose-related decrease in the number of responses under the DRL schedule, mixed results were reported for the number of reinforcers earned. Few of those studies presented a very sophisticated analysis of the data on the IRT frequency distribution. For instance, the IRT data reported by two previous studies (3, 27) were categorized into a bin with a longer duration (3 s) instead of a shorter one, and no detailed information was presented for the frequencies of the IRTs beyond the reinforced bin. Thus, it was difficult to determine how neuroleptic drugs affected DRL behavior. Nevertheless, those previous data regarding the effects of neuroleptics on DRL behavior were argued to have been derived from dopamine receptors being blocked by these drugs. The authors thus thought that it would be interesting to determine which subtypes of dopamine receptors are involved in this effect, since dopamine receptors are now well-classified into D_1 and D_2 families (7, 44, 45). Surprisingly, the acute effects of selective dopamine receptor antagonists on DRL behavior have apparently not yet been examined.

If the effects of drugs on the DRL behavioral performance are to be directly compared, it is important to maintain the parameter of the required duration of the DRL task at an identical value. The present study compared the acute effects of d-amphetamine on a DRL 10-s task with those of selective dopamine D_1 and D_2 receptor antagonists (SCH23390 and raclopride, respectively). For reference, pentylenetetrazole and ketamine were evaluated in order to certify that behavioral changes on the DRL task induced by d-amphetamine were specifically derived from the pharmacological actions of psychostimulants. Pentylenetetrazole is known as a nonspecific motor stimulant drug which acts on the central nervous system, while ketamine is a phencyclidine (PCP)-like stimulant with abuse potential (11).

Materials and Methods

Subjects

Subjects were male Wistar rats, averaging approximately 250 g of body weight upon receipt (obtained from the Breeding Center of Experimental Animals, National Taiwan University Hospital, Taipei). After 10 days of adaptation with food and water provided *ad libitum*, rats were maintained on a water-deprivation regimen such that 5 min access to tap water in the home cage occurred no sooner than 30 min after the end of each daily experimental session. The rats were monitored and kept at 85% of their pre-deprivation body weight. Food pellets were continuously available in each home cage. Training and/or test sessions were conducted daily at the same time (10:00 to 15:00) each day during the light portion of the vivarium's 12/12-h light/dark cycle. The temperature of the colony was maintained at $23\pm1°C$ throughout the experiment. The subjects were about 130 days old on the beginning of drug treatment. The Committee of Experimental Animal Use and Care of National Cheng-Chi University approved all procedures applied in the present study, and treatment of rats complied in all respects with the Taiwanese Psychological Association's ethical standards for the use of animals in research.

Apparatus

Operant responses were measured in two chambers (MED Associated, St. Albans, VT, USA) located in a room separated from the animal colony. The interior dimensions of each chamber were 20×25×30 cm. Aluminum panels formed the front and back walls, and clear Plexiglas comprised the remaining sides and top. Stainless steel rods (with a diameter of 5 mm) were set 11 mm apart to provide flooring. Each chamber was equipped with a lever placed 4 cm above the floor and positioned 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 (tap water) delivery mechanism contained 0.2 ml water for each presentation. The water was

delivered into a receiving dish located on the center of the front panel and 4 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. The chamber was enclosed in a plywood box with a fan to provide the necessary ventilation and masking noise. Two operant chambers were serviced and controlled by a microcomputer. The contingency for schedules of reinforcement was programmed and compiled *via* a commercial kit (Medstate Notation) from MED Associated. An in-house design program, written in Visual Basic (version 6.0 for Windows 98; Microsoft), was applied for data reduction for the IRTs (see below).

Drugs

d-Amphetamine sulfate (0.5 and 1.0 mg/kg), raclopride l-tartrate (0.2 and 0.4 mg/kg), and pentylenetetrazole (5 and 10 mg/kg) were purchased from Sigma (St. Louis, MO, USA). SCH23390 hydrochloride (0.05 and 0.1 mg/kg) was obtained from Tocris Cookson (Bristol, UK). Ketamine hydrochloride (1 and 10 mg/kg) was received from Sin-Tung Chemical (Tao-Yuan, Taiwan). Prior to the injection, all drugs were freshly prepared by being dissolved in 0.9% physiological saline. During drug test days, injections of the drug or vehicle were administered intraperitoneally (IP) at a constant volume of 1 ml/kg of body weight, except at 2 ml/kg for ketamine. SCH23390 and raclopride were injected 1 h before commencement of a behavioral session, whereas d-amphetamine and pentylenetetrazole were injected 15 min prior to testing, and ketamine was administered 30 min before the start of behavioral testing. Doses of d-amphetamine were chosen based on previous reports indicating the least potential to induce stereotypic responses (18). The dose ranges applied for SCH23390 and raclopride had a low potential to produce active immobility or catalepsy as referenced to previous work from this and other laboratories (12, 13, 21-23, 33, 43).

Procedures

Each subject initially received two sessions of magazine training, at which time the rat learned to drink water from the receiving dish inside the operant chamber. Subjects were then manually shaped to press the lever on a continuous reinforcement schedule, the so-called fixed-ratio 1 (FR1) schedule. Each rat was then subjected once a day to a 15-min operant session, always in the same chamber. Subjects were trained to respond on a FR5 schedule of reinforcement for 5 sessions. Afterwards, their responses were maintained on a DRL schedule of reinforcement in

which a reinforcer was delivered contingent upon a lever press if at least 5 or 10 s had elapsed since the previous press. Premature responses led to a non-reinforcement contingency and a resetting of the interval delay, as indexed by the non-reinforced response. Each lever press, whether reinforced or not, reset the delay timer. Subjects had been trained on the DRL 5-s schedule for five to seven sessions before beginning the DRL 10-s training. The criterion for definition of a stable baseline was less than 10% variation in the response rate for three consecutive sessions. There were 54 sessions, on average, of DRL 10-s behavioral training before stable performance was achieved. All drug treatments were conducted thereafter on DRL 10-s task.

Eighteen subjects were assigned to receive d-amphetamine challenge under between-subject design (n=6 for each dosing group), and twelves subjects under within-subject design received drug treatments of SCH23390 and raclopride in that order. An additional two groups (n=10 each), both under within-subject design, were employed to test penty-lenetetrazole and ketamine, respectively. Except d-amphetamine, the dose-response effects of four other drugs were determined in a counterbalanced manner. Treatment with each dose of drug was always conducted when the rats had maintained a stable DRL 10-s performance. In other words, each dosing injection day was preceded by at least three non-injection training sessions.

Data Collection and Analysis

The following data were collected from each session for each rat: numbers of total responses, reinforced responses, and non-reinforced responses, as well as the IRT (the time elapsed between each response). The IRT data were further plotted into a distribution with response frequencies for 21 consecutive 1-s time bins. A bimodal IRT distribution was reliably shown at the baseline stage as well as the vehicle control. Quantitative analyses of the IRT distribution included burst responses, peak time, and mean peak rate. The burst responses were the summed frequencies of IRTs of less than 3 s (bins 1 and 2 as shown in Figs. 2 to 4). The peak time and mean peak rate were calculated from the de-burst IRTs, in which a moving average based on four consecutive 1-s bins was applied to smooth the distribution. With the maximum frequencies of a 4-s epoch identified, the peak time was the mean value of the IRTs that fell within those four bins. The mean peak rate was calculated by the summed frequencies of those four bins divided by four.

Statistical analyses were conducted using commercial software (Statistica version 5.5, Statsoft,

Tulsa, OK, USA). Dependent measures were subjected to separate one-way repeated analyses of variance (ANOVAs), with the dose as the within-subject factor. If the overall F score was significant, the Scheffe test was used for *post hoc* comparisons. A significance level was set at $P < 0.05$ for all tests. Results are presented as the mean with the standard error.

Results

Stabilized subjects responded at efficiencies of 41%~51% (number of reinforced responses ÷ number of total responses) at the baseline stage. This type of stable performance was always maintained before each drug treatment was carried out. In Figure 1, the total number of responses, the non-reinforced responses, and the reinforced responses are displayed for treatments with d-amphetamine, SCH23390, and raclopride, respectively. As shown in the top panel of Figure 1, d-amphetamine significantly increased the non-reinforced responses ($F(2, 15)=5.538$, $P < 0.05$) and decreased the reinforced responses ($F(2, 15)=9.446$, $P < 0.01$) in a dose-related fashion. *Post hoc* comparisons of the non-reinforced responses indicated that a significant dose effect was derived from the difference between the vehicle control and high-dose treatments ($P < 0.05$). Regarding reinforced responses, *post hoc* comparisons revealed that both dose treatments significantly differed from the vehicle control (both $P < 0.01$). Although an increasing trend was observed for the total number of responses, it was not statistically significant. The results of ANOVA for this test revealed only a marginal significance ($F(2, 15)=3.331$, $p=0.0635$). As shown in the middle panel of Figure 1, SCH23390 produced significant dose-related decreases in the total number of responses and the non-reinforced responses, ($F(2, 22)=31.947$ and $F(2, 22)=28.645$, both $P < 0.001$). For either variable, *post hoc* comparisons revealed that both dose treatments significantly differed from the vehicle control (both $P < 0.001$). The reinforced responses were not significantly affected by SCH23390 ($P > 0.1$). As shown in the bottom panel of Figure 1, raclopride significantly reduced the responses of all three variables ($F(2, 22)=43.51$ for the total responses, $F(2, 22)=13.422$ for the non-reinforced responses, and $F(2, 22) = 31.963$ for the reinforced responses, all $P < 0.001$). *Post hoc* comparisons indicated that total responses and non-reinforced responses of both dose treatments significantly differed from the control (both $P < 0.001$), while only the reinforced responses of the high-dose treatment significantly differed from the control ($P < 0.001$).

As can be seen from Figs. 2A, 3A, and 4A, subjects treated with the saline vehicle consistently yielded two peaks, one for the burst responses and
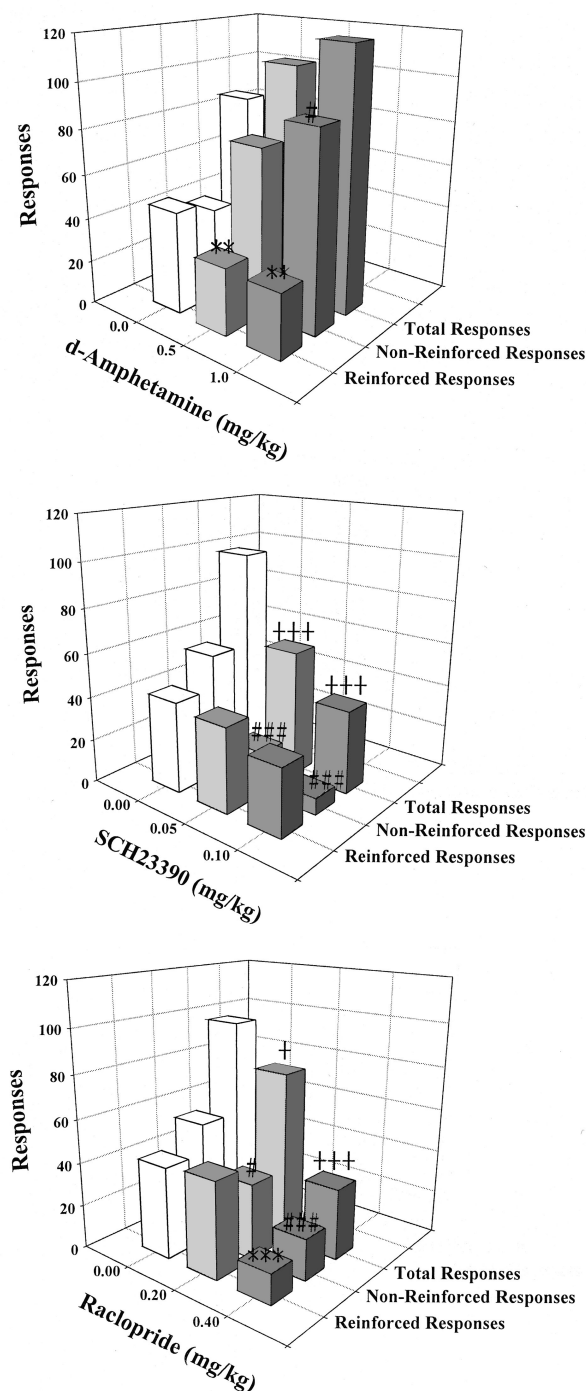

Fig. 1. Acute dose effects of d-amphetamine (top), SCH23390 (middle), and raclopride (bottom) on three dependent variables including total responses, non-reinforced responses, and reinforced responses measured for a DRL 10-s behavioral task. Pluses denote significant differences from the vehicle control on total responses identified by Scheffe tests following one-way ANOVA: $^{+}P < 0.05$ and $^{+++}P < 0.001$. Correspondingly, $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ denote significant differences from the vehicle control on non-reinforced responses, and $^{**}P < 0.01$ and $^{***}P < 0.001$ denote significant differences from the vehicle control on reinforced responses.
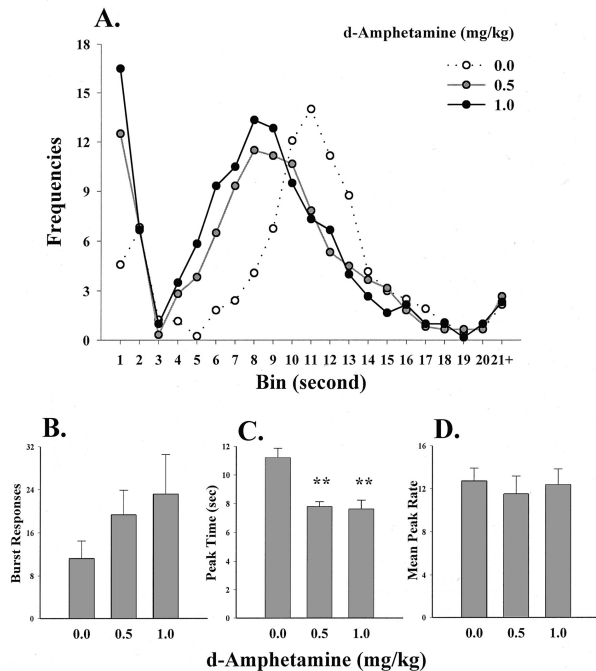
Fig. 2. Effects of d-amphetamine on the distribution of inter-response times (IRTs) and three relevant measures for a DRL 10-s behavioral task. Panel A presents IRT data as the mean frequency of IRTs on each 1-s bin up to 21 s and greater. Representative results for the quantitative analyses of the IRT distribution as measured by burst responses, peak times, and mean peak rates are shown in the three bottom panels (B~D). Asterisks denote significant differences from the vehicle control as identified by Scheffe tests following one-way ANOVA: **$P < 0.01$.
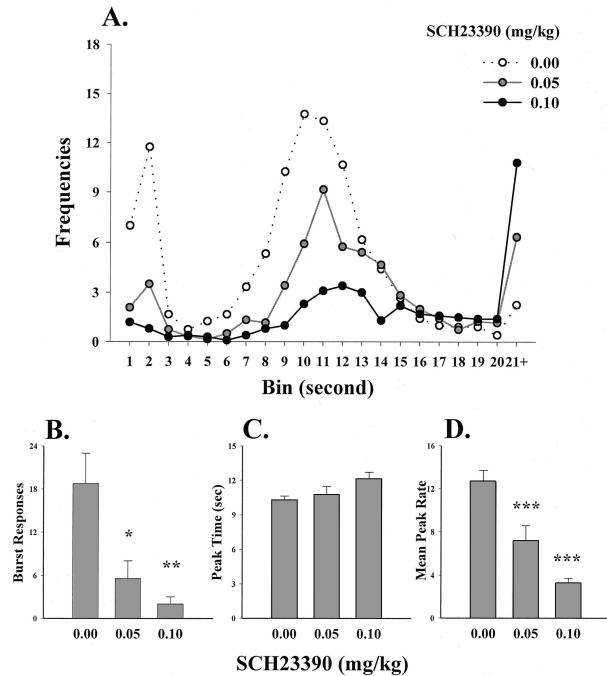

Fig. 3. Effects of SCH23390 on the distribution of inter-response times (IRTs) and three relevant measures for a DRL 10-s behavioral task. Other details are given in Fig. 2. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences from the vehicle control.

another one close to the schedule criterion (10 s in this study). Figure 2 presents the effects of d-amphetamine on the IRT distribution with quantitative analyses of burst responses, peak times, and mean peak rates as shown in the bottom three panels, respectively. As shown in Figure 2A, d-amphetamine appeared to elevate the height of the peak of burst responses and shift the other peak of the de-burst responses horizontally to the left on the IRT distribution. While the elevation of the burst responses was not statistically significant (Figure 2B), the leftward shift of the de-burst responses was significantly confirmed by the drug-decreased peak time as shown in Figure 2C ($F(2, 15)=13.515$, $P < 0.001$). *Post hoc* comparisons indicated that the peak times of both dose treatments differed from the control (both $P < 0.01$). In contrast to the peak time, the mean peak rate was not significantly affected by d-amphetamine (Figure 2D).

Figure 3 presents the IRT data and analyses for SCH23390 treatment. The bimodal IRT curve under the vehicle condition was dispersed under SCH23390 treatment by significant suppression of the burse responses ($F(2, 22)=9.531$, $P < 0.01$) and mean peak rate ($F(2, 22)=31.314$, $P < 0.001$). *Post hoc*

comparisons indicated that the burst responses and the mean peak rates of both dose treatments differed from the control. The increasing trend of the peak time produced by SCH23390 was not statistically confirmed.

Figure 4 presents the IRT data and analyses for raclopride treatment. Similar to SCH23390, raclopride flattened the IRT distribution by decreasing the burst responses ($F(2, 22)=4.794$, $P < 0.05$) and the mean peak rate ($F(2, 22)=54.377$, $P < 0.001$). *Post hoc* comparisons indicated that the mean peak rates of both dose treatments differed from the control, while only the burst responses of the high-dose treatment differed from the control. In addition, raclopride significant decreased the peak time ($F(2, 22)=6.737$, $P < 0.01$). This significance was mainly derived from the difference between the high dose and the control ($P < 0.05$).

As shown in Figure 5, neither pentylenetetrazole nor ketamine produced significant alteration in the IRT distribution. None of the ANOVA results indicated a significant dose effect for any of the six dependent variables for pentylenetetrazole or ketamine (data not shown).

Discussion

The present results show that acute systemic

A.

Raclopride (mg/kg)
- ·o· 0.0
- 0.2
- 0.4

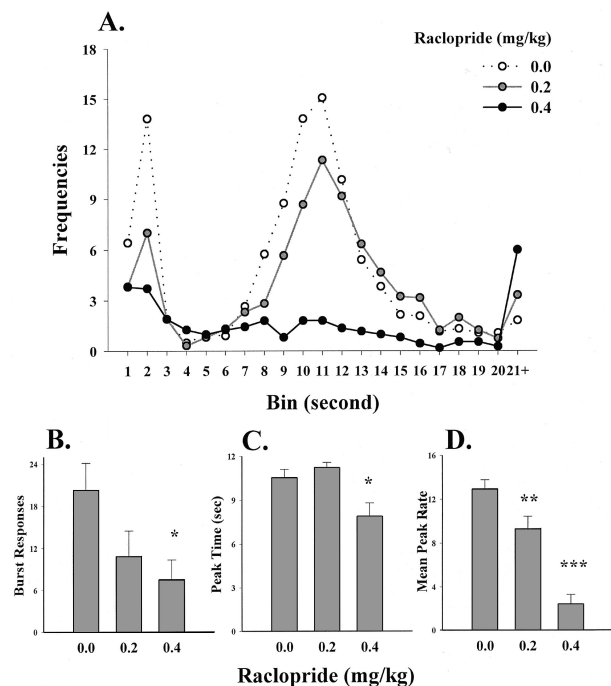## B.   C.   D.

Raclopride (mg/kg)

Fig. 4. Effects of raclopride on the distribution of inter-response times (IRTs) and three relevant measures for a DRL 10-s behavioral task. Other details are given in Fig. 2. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences from the vehicle control.

Pentylenetetrazole (mg/kg)
- ·o· 0
- 5
- 10

Ketamine (mg/kg)
- ·o· 0
- 1
- 10

Fig. 5. Effects of pentylenetetrazole (upper panel) and ketamine (lower panel) on the distribution of inter-response times (IRTs) on a DRL 10-s behavioral task.

injection of d-amphetamine profoundly affects the operant performance of rats under a DRL schedule by increasing the response rate and decreasing the reinforcement rate. Based on quantitative analyses of the IRT data, the present study further reveals that d-amphetamine caused a horizontal leftward shift of the IRT distribution at the doses used. Such a shifting effect was confirmed by a significant decrease in the peak time, with no dramatic changes in the mean peak rate or burst response. The alteration of the DRL behavior produced by d-amphetamine can be highly specific to its drug actions as a psychostimulant, because no such disruptive effect was observed with challenge by the two reference drugs, pentylene-tetrazole and ketamine. Neither agent produced a significant effect on the DRL response. Furthermore, differential effects were obtained from comparing the dose-response functions of SCH23390 and raclopride with those of d-amphetamine. Gross responses to the present DRL task were inhibited by these two selective dopamine receptor antagonists, as shown by the significant dose-related decreases in the total, non-reinforced, and burst responses.

Not unexpectedly, the increment of the response rate and the decrement of the reinforcement rate produced by d-amphetamine reported herein are in agreement with findings of most previous reports
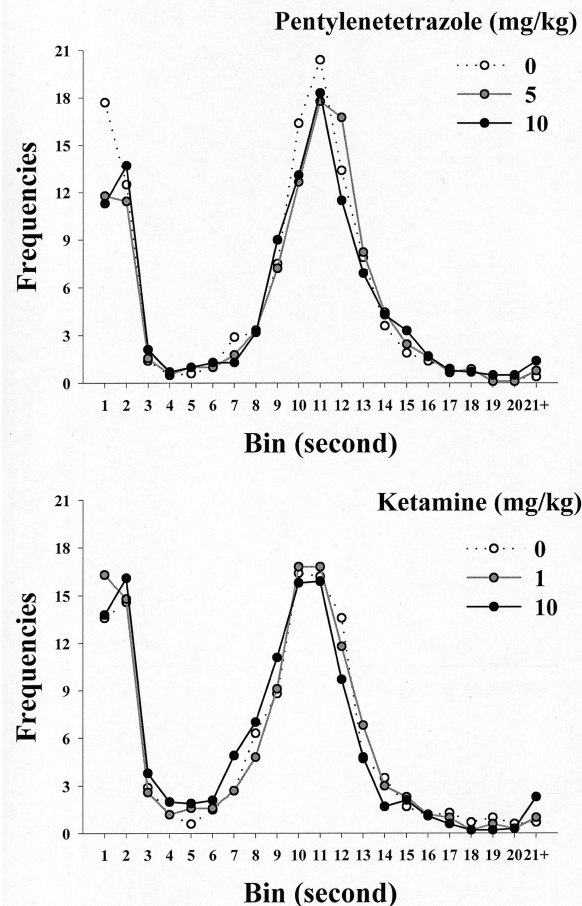
(1-3, 5, 6, 34, 36, 38, 39, 41, 50). Despite the duration requirement set for DRL tasks in these studies that variously ranged from 10 to 72 s, these gross changes in the DRL responses were consistently and markedly induced by the systemic injection of d-amphetamine in the rat. However, it should be noted that this statement is generally true when d-amphetamine is administered at low to moderate doses (i.e., < 2.5 mg/kg) but not when this drug is given at higher doses with the potential to induce stereotypic behavior (39, 41, 49). The response rate increase induced by d-amphetamine may also be a drug effect specifically linked to DRL behavior as compared to that decreased by this agent on other types of operant behavior such as the fixed ratio (FR). In accordance with the rate-dependency principle (9), the change in the response rate by d-amphetamine relies on the baseline rate of a certain type of schedule-controlled (operant) behavior. That is, amphetamine tends to increase the response rate when baseline rates are low, and vice versa to decrease the response rate when baseline rates are high. In regard to the response rate changed by

d-amphetamine in the present study, a significant decrement was shown for the variable of non-reinforced responses (the top panel of Figure 1). The increasing effect on the total number of responses was only marginally significant. This effect may have reached statistical significance if the study had used a greater sample size or had administered a slightly higher dose of d-amphetamine. Nevertheless, the significant increase in the non-reinforced responses obtained in the present study is consistent with the general profile of d-amphetamine's effect on DRL behavior, in this case, the response rate.

Results of the horizontal leftward shift of the de-burst IRT distribution produced by d-amphetamine at the test doses were consistent with previous reports using rats treated with the same drug and maintained on DRL 10-s (50), 15-s (41), 20-s (34), 36-s (38), or 72-s schedules (1, 36). Although this IRT leftward shift is a remarkable effect produced by d-amphetamine across a wide range of different durations required for reinforcement contingency of DRL tasks, only a few of those previous studies conducted quantitative analyses of this phenomenon. According to the so-called peak deviation analysis, d-amphetamine dose-dependently decreased the peak location and peak area (1, 36, 38). These two decreased metrics under d-amphetamine treatment are congruent with the decreased peak time reported here for the same drug. This solid effect of a peak shift to the left on the de-burst IRT distribution induced by d-amphetamine is suggested to be predominately mediated by a neurochemical reaction of brain dopamine (38). In addition to the direct comparison between the effects of the d-amphetamine and dopamine receptor antagonists reported herein, it may be informative to collect data from an experiment manipulating the pharmacological interactions between these compounds.

Among other possibilities, our emphasis on a specific psychological process which is involved in temporal regulation may have contributed to the aforementioned leftward shift in the de-burst IRT distribution. On a behavioral level, d-amphetamine-treated subjects responded before adequate time had elapsed to allow the response to be reinforced. In other words, d-amphetamine caused an overestimation of the rate of time passage. This statement is supported by a recent study using a modified DRL task. Wiley and associates (51) employed a multiple signaled-unsignaled DRL 15-s schedule to separate the timing process and behavioral inhibition in rats treated with d-amphetamine. The leftward shift in the mean IRT produced by this drug appeared only during the unsignaled components, suggesting that the predominant effect of d-amphetamine is on timing and not on behavioral inhibition. Further evidence

supporting this argument is based on the findings from previous studies that measured different timing processes of operant tasks other than DRL. For example, the peak interval procedure and temporal bisection methods have been used to test time-estimation ability for isolating clock speed from other potential factors. Acute treatment with amphetamine has consistently been demonstrated to decrease the peak time in the peak interval procedure and the point of subjective equality in the temporal bisection method in animals (4, 20, 25, 26, 30). Accumulating data indicates that this directional change in the duration response distribution under acute amphetamine treatment is due to acceleration of the subject's clock speed produced by the drug's activation of the brain dopamine system (32).

In terms of neuropsychopharmacology, the involvement of dopamine in timing has been supported by deceleration of the clock speed under non-selective dopamine receptor antagonists. For instances, haloperidol produced rightward shifts on the peak time in the peak procedure (4, 10) and on the psychophysical function of the temporal bisection procedure (25, 31). Although dopamine D_2 receptors are inferred to play an important role in affecting the clock speed for time estimation (31, 32), it is impossible to characterize the involvement of dopamine D_1 and D_2 receptor subtypes in timing behavior without using selective dopamine agents. Frederick and Allen (14) reported the acute effects on the peak interval procedure of the selective actions of dopamine agonists and antagonists on D_1 and D_2 receptors. The results showed significant decreases in peak times produced by both D_1 and D_2 receptor agonists (SKF38393 and quinpirole, respectively). In contrast, D_1 and D_2 receptor antagonists (SCH23390 and eticlopride, respectively) increased the peak times despite these antagonist effects not reaching a significant level. Compatible results reported by a recent study of Drew and associates (10) showed that SCH23390 failed to affect timing processing on a two-interval peak procedure as compared to significant effects produced by haloperidol. Thus, the lack of SCH23390-produced alteration in the peak-interval procedure indicates that D_2 receptor blockade alone is not adequate to affect timing behavior. With the profound effects produced by haloperidol, a mixed D_1/D_2 receptor antagonist with higher affinity for D_2 receptors, D_2 receptors are more likely involved in modulating the clock speed of timing (31, 32). Together, it is possible that drug acting on the central dopaminergic system affects the clock speed of the timing process involved in the present DRL task.

The acute effects of SCH23390 and raclopride showed different profiles of behavioral performance on the present DRL task compared with those of d-

amphetamine. These two dopamine antagonists produced overall suppressant effects on all four distinct response-rate related variables measured in the present study. However, unexpectedly, the peaks on the drug-treated IRT curves were not shifted rightward at all. This part of the results indicates that both drugs tested at the current doses generally affect behavioral outputs rather than specifically disrupting temporal regulation of DRL tasks. As such, the ineffectiveness of selective dopamine receptor antagonists to alter the peak time has been reported from other studies using the peak interval task (10, 14). Although a significant decrease in peak time was detected with the present treatment of raclopride given at the high dose, it would be more conservative to draw a link between the temporal dysfunction of DRL behavior and D_2 receptor antagonism due to the relatively less IRT information collected from that drug treatment. Nevertheless, the decrease in total responses produced by both SCH23390 and raclopride herein is consistent with previous studies using DRL tasks with various duration parameters to test a variety of non-selective dopamine receptor antagonists in the rat as described earlier. Furthermore, no significant differential effect between SCH23390 and raclopride was revealed for other measures of the response rate used in the present DRL task. Such results indicate that sole blocking of either D_1- or D_2-typed dopamine receptors can impair behavioral performance maintained on a DRL schedule.

In contrast to the suppression of the burst response produced by either SCH23390 or raclopride, an increasing trend of the burst response was observed under d-amphetamine treatment. However, this increment was not statistically confirmed. Although the burst response increased by amphetamine has been addressed in qualitative terms by most previous studies, only a few of them quantitatively analyzed this part of the data (2, 34, 36, 38). Unfortunately, there has been a lack of consistent results from both ways of elucidating the effect of amphetamine on the burst response. Those mixed results of the effects of amphetamine on the burst response as reported by those studies can be attributed to the diverse experimental manipulations among studies, including the drug dose, the duration required for DRL reinforcement contingency, and the behavioral history of training prior to the baseline measurement. With the same administration and doses of d-amphetamine as used in the present study, Bizot (2) reported a negative result for the burst response, identical to what we found. As the bimodal nature of the DRL IRT distribution consists of burst and de-burst parts, it is possible that different mechanisms are involved in behavioral responses for these two categories. To incorporate the de-burst IRTs discussed earlier, it

seems likely that the current treatment with d-amphetamine affected the temporal regulation rather than the inhibitory control of the DRL 10-s task.

Regarding the lack of a disrupting effect on DRL behavior observed for pentylenetetrazole and ketamine, one might argue that the doses of these two compounds used in the present study were not adequately potent. This speculation might not be true by considering the following evidence from other studies administering the same doses of both compounds to restrict the induction of psychotropic actions. First, the lack of any seizures observed in the subjects treated with pentylenetetrazole here apparently confirms that the doses of this drug applied in the present study are subconvulsant. Systemic injection of pentylenetetrazole at doses of 10 ± 5 mg/kg is effective as a discriminative stimulus for rats trained in drug discrimination tasks (15). Pentylenetetrazole given in the aforementioned dose range significantly inhibits the discriminative properties of alprazolam, an unusual anxiolytic and antidepressant agent (17). Moreover, a unique type of operant behavior established by training the rat to press a lever to terminate a negative reinforcing stimulus induced by electrical stimulation of the periaqueductal gray was significantly suppressed by pentylenetetrazole at a dose of 10 mg/kg (19). Second, in regard to ketamine, the reward property of this agent has been reported in rodents using drug discrimination and self-administration paradigms (35, 37). Rats were capable of discriminating 5 and 10 mg/kg ketamine from saline and of self-injecting this drug with 1.1 mg/kg per infusion. Ketamine given at 10 mg/kg, the same as the high dose applied in this study, significantly decreased the response rate to 35% of the control in approximately of either a fixed-ratio (FR) or fixed-interval (FI) component in a multiple FI 300-s and FR 30 schedule in the rat (29). Third, in addition to behavioral measures which rely upon the operant paradigm as described above, the conditioned place preference often used to measure the effects of a drug reward has been demonstrated in rats treated with pentylenetetrazole or ketamine. While d-amphetamine given at doses ranging from 0.5 to 2 mg/kg (IP) can reliably induce a conditioned place preference (22, 46), similar place conditioning effects have been shown under the same systemic injection of either pentylenetetrazole given at 10 mg/kg (16) or ketamine given at 3 and 10 mg/kg (47). Thus, failure to affect DRL behavior under pentylenetetrazole or ketamine treatment in the present study was unlikely due to inadequate dosing. Conversely, this observation from pentylenetetrazole and ketamine provides evidence to support the idea that alteration of DRL performance by d-amphetamine is specifically derived from its psychostimulant effect

rather than a general CNS stimulant action or solely due to the abuse potential.

In conclusion, the present study investigated the acute effects of d-amphetamine on the operant performance of DRL 10-s behavior as compared to SCH23390, raclopride, pentylenetetrazole, and ketamine. d-Amphetamine remarkably increased non-reinforced responses and decreased reinforced responses, with a subtle but insignificant increase in the burst response. The profound increase in the non-reinforced responses leading to a leftward shift in the de-burst IRT distribution produced by d-amphetamine highlights alteration of the temporal regulation of DRL tasks by this drug. The absence of such behavioral changes observed from the two reference drugs, pentylenetetrazole and ketamine, supports the argument for a specific interaction between DRL behavior and the psychostimulant drug. In addition, differential effects between d-amphetamine and selective dopamine receptor antagonists suggest that distinct neuropharmacological mechanisms on dopaminergic synapses are involved in the effects of these agents on DRL behavior in distinctive profiles. The idea about the peaks shifting in opposite directions on the IRT distribution of DRL behavior for d-amphetamine and dopamine antagonist(s) is more complicated than what was earlier thought.

Acknowledgments

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