

# Dopamine Receptor Antagonists Reverse Amphetamine-Induced Behavioral Alteration on a Differential Reinforcement for Low-Rate (DRL) Operant Task in the Rat

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## Abstract

Previous studies have shown that amphetamine significantly alters operant responding on the behavior maintained on a schedule of differential reinforcement of low-rate (DRL). As such, behavioral deficiency of DRL responding has been observed by the drug-induced increase of non-reinforced responses and a leftward shift of inter-response time (IRT) curve on DRL responding in the rat. However, the neurochemical basis for amphetamine-induced DRL behavioral alternations remain to be elucidated. The present study was then designed to examine whether the effects of amphetamine were dependent on dopamine-subtyped receptors, this was carried out by the co-administration of the selective D1 and D2 receptor antagonists, SCH23390 and raclopride respectively. Rats were first trained to perform on DRL 10-sec task and then divided into four groups, which received separate types of double injections before the behavioral session. The four groups were the saline control group, the amphetamine alone group, the dopamine antagonist alone group, and the combination amphetamine and dopamine antagonist group. The saline control group performed DRL responding in an efficient manner with a major index for the peak time of the IRT curve, which was fairly localized within the 10-sec bin throughout the test phase. The subjects injected with amphetamine (1 mg/kg) significantly shortened IRT that led to a leftward shift of IRT curve, which was further revealed by a decreased peak time without significant effectiveness on the peak rate and burst response. Even though the group given SCH23390 or raclopride alone showed profound disruption on DRL behavior by flattening the IRT curve, the co-administration of amphetamine with SCH23390 or raclopride reversed the aforementioned amphetamine-induced behavioral deficiency on DRL task. Together, these results suggest that the dopamine D1 and D2 receptors are involved and important to the temporal regulation of DRL response under psychostimulant drug treatment. Furthermore, this highlights the involvement of the brain dopamine systems in the temporal regulation of DRL behavior performance.

**Key Words:** D1 and D2 receptors, IRT analysis, psychostimulant, raclopride, SCH23390, timing behavior

## Introduction

Considerable experimental evidence indicates that amphetamine has complex behavioral effects (11). At the behavioral level, among many types of behavioral measures, an operant task “differential reinforcement for low-rate responding (DRL)” has been used to

examine behavioral inhibition and temporal regulation under amphetamine treatment. Rats trained on a DRL schedule of reinforcement are required to inhibit or withhold responding for a minimum specified period of time in order to obtain a reinforcer. Accumulated data show that acute treatment with amphetamine at moderate doses (0.5 ~ 3.0 mg/kg) results in the disruption

of DRL behavioral performance by increasing the number of responses and decreasing the number of reinforcers obtained (2, 3, 5, 7, 21, 24, 26, 27, 29, 34). In addition to the measurement of the aforementioned responses and reinforcement rates, the inter-response time (IRT) specified for each response is now recognized as a useful measure when evaluating drug effects on DRL behavioral performance. A limited number of studies have reported IRT data using qualitative analysis to address the way that amphetamine alters DRL behavior (16, 19, 24). Amphetamine, in general, causes rats to respond with shorter IRTs leading to a leftward shift in the IRT frequency distribution. Presumably, this is due to a certain form of neurochemical changes that underlies the amphetamine-induced behavioral alternations in DRL task. Despite the neural effects of amphetamine being complex (30), it has been suggested that these behavioral changes in the DRL schedule produced by amphetamine are predominantly mediated by dopamine released in the brain (26). Thus, it might be expected that dopamine receptor antagonists could block the effects of amphetamine for a specific DRL behavior. However, surprisingly, there has been no study testing the effects of amphetamine in combination with specific dopamine receptor antagonist done on DRL behavior.

The purpose of the present study was to examine further the pharmacological specificity of dopamine sub-typed receptors on the action of amphetamine on DRL behavior. Of particular interest was the determination of which subtypes of dopamine receptors, if any, are involved in this effect, since dopamine receptors are classified into D1 and D2 families (9, 31, 32, 36). SCH23390 and raclopride are known as the selective dopamine D1 and D2 receptor antagonists, respectively, and were used here for this attempt.

## Materials and Methods

### *Subjects*

The subjects were 32 male Sprague-Dawley rats, averaging approximately 250 g of body weight upon receipt from the Breeding Center of Experimental Animals, National Taiwan University Hospital, Taipei. After 10 days of adaptation to food and water provided *ad libitum*, the 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  $23 \pm 1^\circ\text{C}$  throughout the experiment. The subjects were about 130 days old at 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 the treatment of the rats complied in all respects with the Taiwanese Psychological Association's ethical standards for the use of animals in research.

### *Apparatus*

Behavioral measures were conducted in a custom-made operant system with four chambers located in a room separate from the animal colony. The interior dimensions of each chamber were  $20 \times 25 \times 30$  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 (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 delivery mechanism contained 0.04 ml of tap water at each presentation. The water was delivered into a receiving dish (25 mm diameter) 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 to mask any outside noise. The four operant chambers were serviced and controlled by a microcomputer. An in-house designed program, written in Visual Basic (version 6.0 for Windows 98; Microsoft), was used to control the operant environment as well as for data collection.

### *Drugs*

D-Amphetamine sulfate (1.0 mg/kg) and raclopride l-tartrate (0.2 and 0.5 mg/kg) were purchased from Sigma (St. Louis, MO, USA). SCH23390 hydrochloride (0.02 and 0.05 mg/kg) was obtained from Tocris Cookson (Bristol, UK). 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) using a constant volume of 1 ml/kg of body weight. SCH23390 and raclopride were injected 1 h before commencement of a behavioral testing, whereas d-amphetamine was injected 15 min before the start of behavioral testing. To prevent any unwanted side effect, the dose of d-amphetamine was chosen based on previous reports and was at a level that had the least potential

to induce stereotypic responses (11). During testing days, double injections *via* the IP route were implemented on different sides of abdominal surface.

### *Procedures*

Each subject initially received two sessions of magazine training, at which time the rat learned to obtain 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. 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 (see below). Premature responses led to a non-reinforcement contingency and a resetting of the interval delay, as indexed by a 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 the definition of a stable baseline was less than 10% variation in the response rate for three consecutive sessions. Stabilized subjects responded at efficiencies of 40% ~ 45% (number of reinforced responses ÷ number of total responses) at the baseline stage. This type of stable performance was always verified before each drug treatment (see below) was carried out.

Four groups ( $n = 8$  each) were used to test the effects of dopamine receptor antagonists on amphetamine-induced behavioral changes on DRL 10-s task. In order to keep the number and volume of injections in constant, every subject received double injections during the drug test sessions. Each of the four groups received a specific set of double injections before the commencement of behavioral session. The first group received saline injections twice served as the vehicle control and was denoted as the SAL-SAL group. The second group received a saline injection and then an amphetamine injection and was denoted as the SAL-AMP group. The third group received an injection of dopamine receptor antagonist and then a saline injection and was denoted as the SCH-SAL (or RAC-SAL) group. The fourth group received an injection of dopamine receptor antagonist prior to an amphetamine injection and was denoted as the SCH-AMP (or RAC-AMP) group. For the third and fourth groups, the doses of dopamine receptor antagonists were given by an order of 0.02 mg/kg SCH23390, 0.05 mg/kg SCH23390, 0.2 mg/kg raclopride, and 0.5 mg/kg raclopride separately over four drug test sessions. The dopamine receptor antagonist treatment for the third group on each of the drug test session was

corresponding matched for the third and fourth groups. There were at least four daily sessions for the re-running of the DRL 10-s task with no injection between the drug sessions, in which the baseline was checked and certified for the subsequent drug test. D-amphetamine at a dose of 1.0 mg/kg was given throughout the drug tests in the second and fourth groups.

### *Data Collection and Analysis*

The following data were collected from each session for each rat: total responses, reinforced responses, and non-reinforced responses, as well as the IRT (the elapsed time between each response). The IRT data were further plotted to obtain a distribution of response frequencies for 21 consecutive 1-s time bins. A bimodal IRT distribution was reliably shown to occur at the baseline stage as well as with the vehicle control. Six dependent variables were assigned to allow quantitative analyses of the IRT distribution included total responses, reinforced responses, non-reinforced responses, burst responses, peak time, and peak rate. The burst responses were the summed frequencies of the IRTs of less than 3 s (bins 1 and 2 as shown in all Figs.). 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 a maximum frequency 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 from the summed frequencies of those four bins divided by four. Statistical analyses were only performed on data from subjects performed motorically intact after injection to complete the test session with adequate responses. There were several cases of missing data (see Table 1) due to a high level of balking or akinesia induced by drug treatment, in which the subjects made less than fifteen lever presses for total responses. The missing data for the SAL-SAL group came from the same subject during the last three drug tests, and this was not caused by the saline treatment itself. Rather, this subject's claws were hurt during the injection conducted for the second test. The hurt state of this rat resulted in the subject not responding to operant manipulandum when being placed into the test chamber after this occasion.

Statistical analyses were conducted using commercial software (Statistica version 5.5, Statsoft, Tulsa, OK, USA). Dependent measures were subjected to separate one-way analyses of variance (ANOVA) with the planned comparisons that conducted between the Saline-Saline group and each of the other three drug-treated groups. A significance level was set at  $P < 0.05$  for all tests. The results are presented as means plus and minus the standard error of the mean (SEM).

**Table 1. The numbers of subjects with mission data under drug treatment**

	Drug test 1	Drug test 2	Drug test 3	Drug test 4
SAL-SAL group	0	1	1	1
SAL-AMP group	1	1	1	1
SCH (or RAC)-SAL group	1	6	5	5
SCH (or RAC)-AMP group	0	0	0	1

## Results

Figure 1 presents the results of the first drug test, in which the effects of 0.02 mg/kg SCH23390 on amphetamine-induced behavioral alterations on DRL 10-s task were evaluated. The top panel shows the IRT distribution, whereas the six panels below represent the corresponding quantitative analyses for the six dependent variables. As can be seen from the top panel, the subjects treated with the saline vehicle consistently yielded two peaks, one for the burst responses and the other one close to the schedule criterion (10 s in this study). D-amphetamine appeared to shift the peak of the de-burst responses horizontally to the left in the IRT distribution. The overall height of IRT curve was lower for SCH23390 given alone, and this was also true for the combination of SCH23390 and amphetamine injections. In terms of the quantitative analyses, compared to the saline vehicle group, the group receiving amphetamine alone significantly increased total responses [ $F(1, 26) = 8.833, P < 0.01$ ], decreased reinforced responses [ $F(1, 26) = 20.314, P < 0.001$ ], increased non-reinforced responses [ $F(1, 26) = 17.216, P < 0.001$ ], and decreased peak time [ $F(1, 26) = 15.579, P < 0.001$ ]. The group receiving SCH23390 alone showed a significant decrease in the dependent variables for total responses [ $F(1, 26) = 13.192, P < 0.01$ ], non-reinforced responses [ $F(1, 26) = 6.359, P < 0.05$ ], burst responses [ $F(1, 26) = 8.415, P < 0.01$ ], and peak rate [ $F(1, 26) = 9.212, P < 0.01$ ]. The group receiving the combination treatment of SCH23390 and amphetamine showed a significant decrease in the dependent variables of burst responses [ $F(1, 26) = 4.634, P < 0.05$ ], and peak rate [ $F(1, 26) = 5.951, P < 0.05$ ].

Figure 2 presents the results from the second drug test, in which the effects of 0.05 mg/kg SCH23390 on amphetamine-induced behavioral alterations on DRL 10-s task were evaluated. As shown on the top panel, injection of amphetamine alone produced a leftward shift of the IRT distribution. While the overall height of IRT curve dropped by SCH23390, the combined treatment of SCH23390 and amphetamine reversed this trend back toward the control level. With respect to the quantitative analyses, as compared to the saline vehicle group, the group received

amphetamine alone significantly decreased reinforced responses [ $F(1, 20) = 43.605, P < 0.001$ ], increased non-reinforced responses [ $F(1, 20) = 11.395, P < 0.01$ ], and decreased peak time [ $F(1, 20) = 31.498, P < 0.001$ ]. The group given SCH23390 alone significantly decreased total responses [ $F(1, 20) = 12.093, P < 0.01$ ], non-reinforced responses [ $F(1, 20) = 6.617, P < 0.05$ ], and peak rate [ $F(1, 20) = 26.612, P < 0.001$ ], but increase peak time [ $F(1, 20) = 8.105, P < 0.01$ ]. Notice that no significant difference on burst response between this SCH23390 treated group and the saline control due to inadequate error variance in statistical comparison. While the above decreases were detectable and significant for the group with a combined treatment of SCH23390 and amphetamine:  $F(1, 20) = 4.593, P < 0.05$  for total responses,  $F(1, 20) = 4.555, P < 0.05$  for burst response, and  $F(1, 20) = 11.088, P < 0.01$ , the peak time was reversed and was close to the control level ( $P > 0.05$ ).

Figure 3 presents the results from the third drug test, in which the effects of 0.2 mg/kg raclopride on amphetamine-induced behavioral alterations on DRL 10-s task were evaluated. As can be seen from the top panel, subjects treated with the saline vehicle consistently yielded two peaks on IRT distribution. D-amphetamine appeared to shift the peak of the de-burst responses horizontally to the left on the IRT distribution. While the overall height of IRT curve was dropped by this raclopride dosing, the combined treatment of raclopride and amphetamine reversed it toward the control level. In terms of the quantitative analyses, as compared to the saline vehicle group, the group received amphetamine alone significantly increased total responses [ $F(1, 21) = 6.344, P < 0.05$ ], decreased reinforced responses [ $F(1, 21) = 22.538, P < 0.001$ ], increased non-reinforced responses [ $F(1, 21) = 10.864, P < 0.01$ ], and decreased peak time [ $F(1, 21) = 26.459, P < 0.001$ ]. The group given raclopride alone significantly decrease total responses [ $F(1, 21) = 5.254, P < 0.05$ ] and peak rate [ $F(1, 21) = 27.688, P < 0.001$ ]. These two decreases were reversed in the group treated with a combination of raclopride and amphetamine, which total responses and peak rate were close to those of the saline control ( $P > 0.05$ ). However, the peak time of this group was still shorter than that of vehicle control group ( $F(1, 21) =$

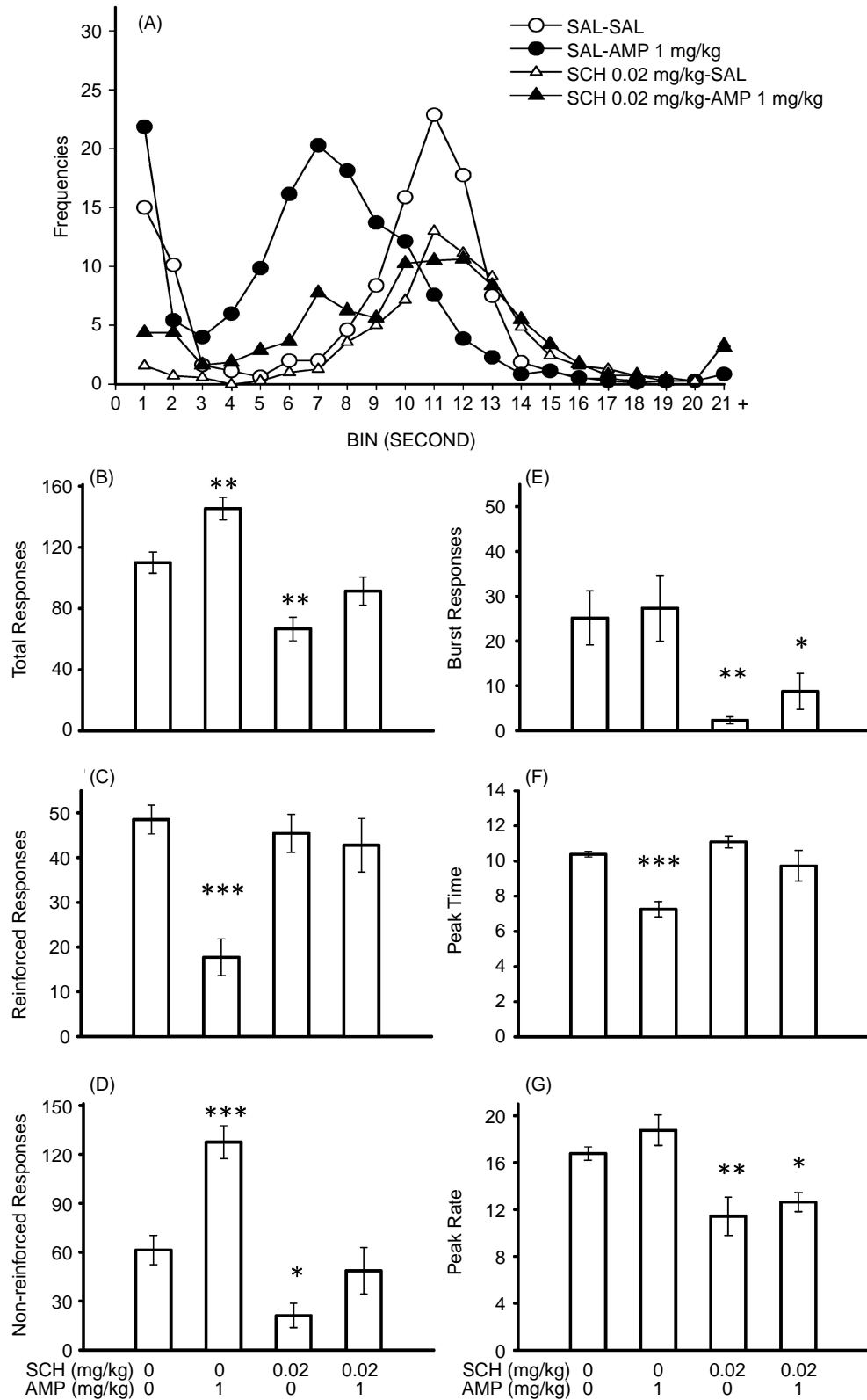


Fig. 1. Effects of 0.02 mg/kg SCH23390 on d-amphetamine-induced behavioral changes on the distribution of inter-response times (IRTs) and six relevant measures for a DRL 10-s task. Panel A presents IRT data as the mean frequency of IRTs for each 1-s bin up to 21 s and greater (represented as 21+). Representative results for the quantitative analyses of the IRT distribution as measured in terms of total responses (B), reinforced responses (C), non-reinforced responses (D), burst responses (E), peak times (F), and peak rates (I) and are shown in the lower six panels. Asterisks denote significant differences between the indicated group and the vehicle control group as revealed by planned comparisons based on one-way ANOVA: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

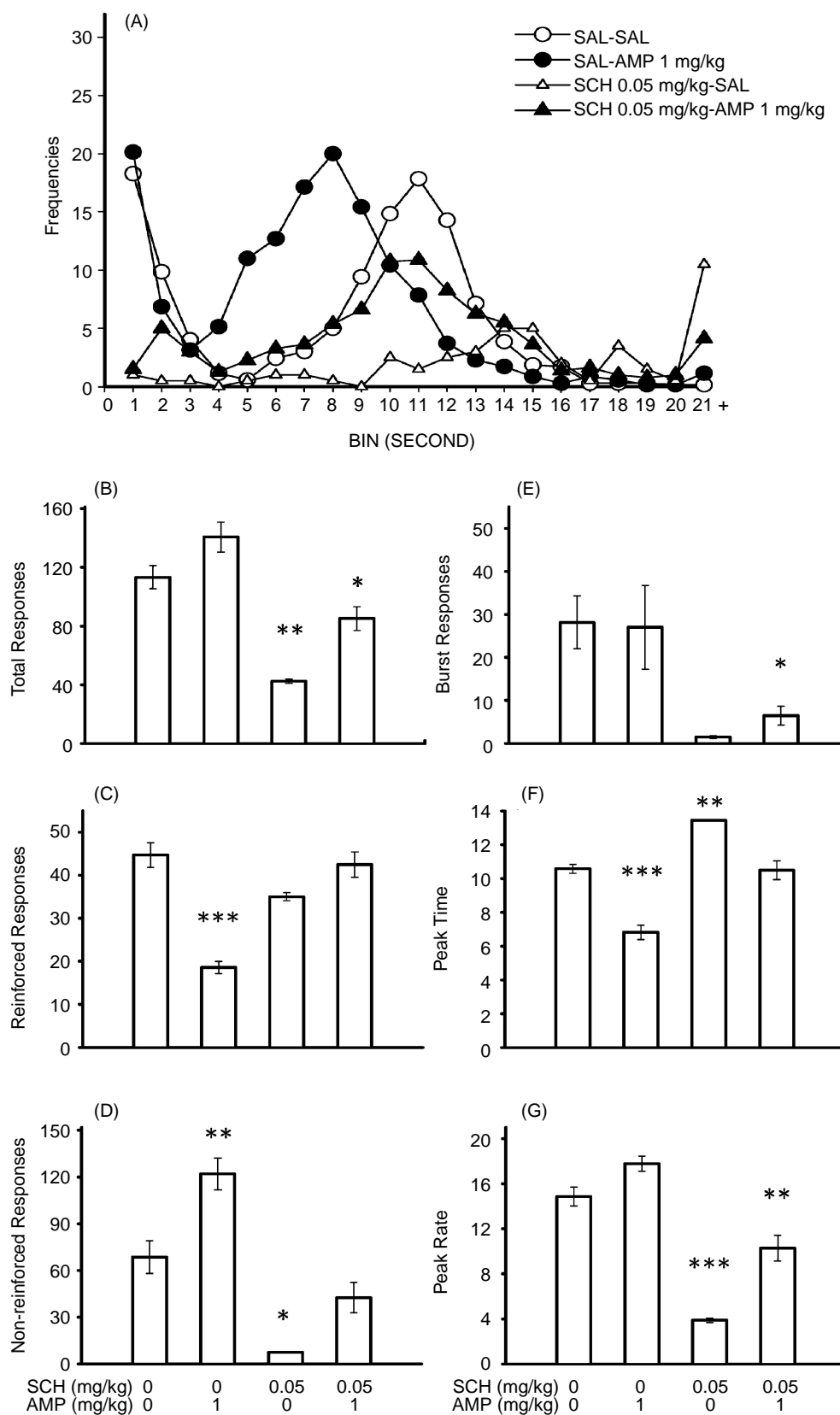


Fig. 2. Effects of 0.05 mg/kg SCH23390 on d-amphetamine-induced behavioral changes on the distribution of inter-response times (IRTs) and six relevant measures for a DRL 10-s task. Other details are as in Fig. 1.

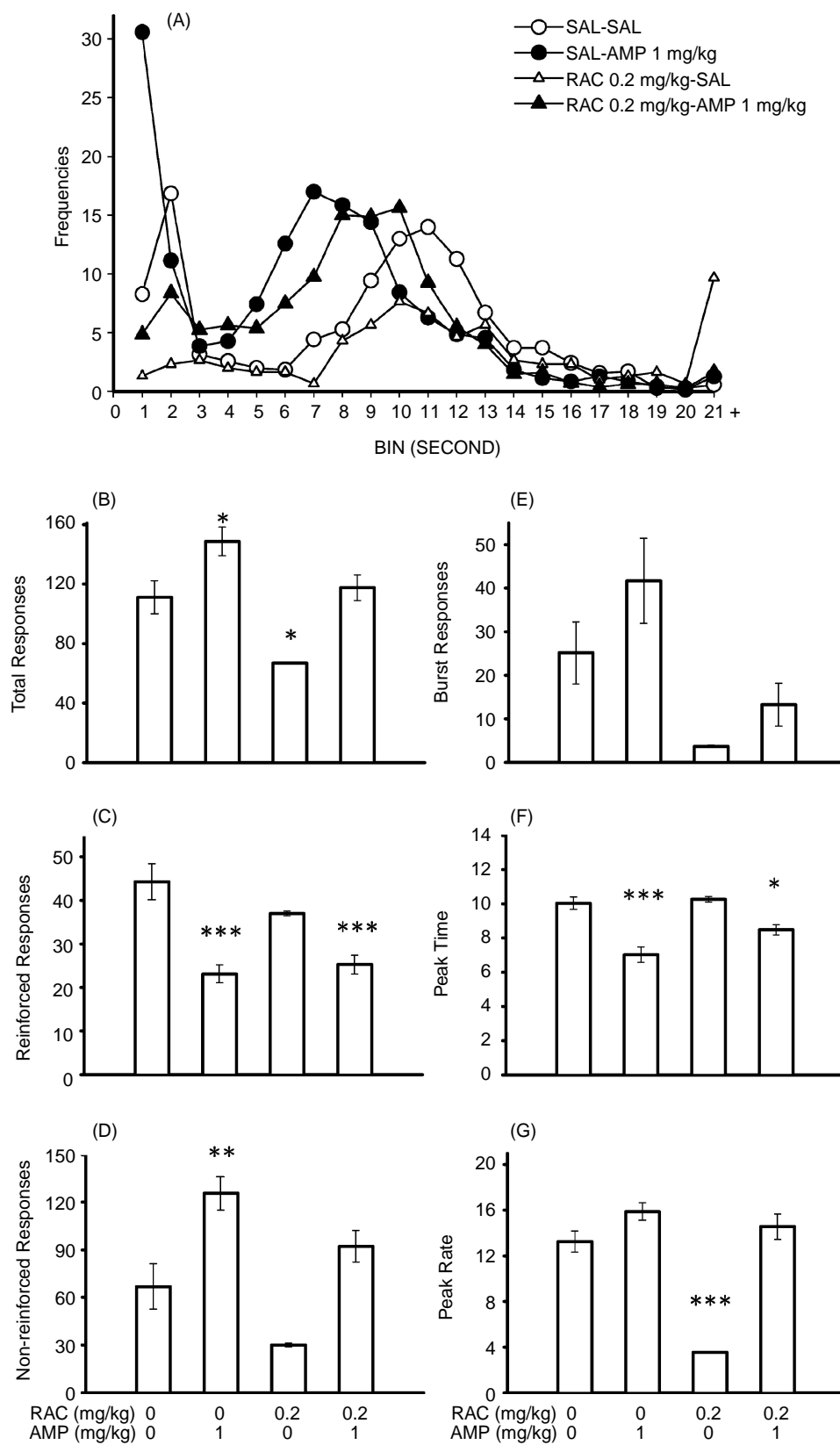


Fig. 3. Effects of 0.2 mg/kg raclopride on d-amphetamine-induced behavioral changes on the distribution of inter-response times (IRTs) and six relevant measures for a DRL 10-s task. Other details are as in Fig. 1.

7.545,  $P < 0.05$ ), so was its lower number of non-reinforced responses [ $F(1, 21) = 19.488$ ,  $P < 0.001$ ]. Notice that no significant difference on burst response between the group treated with raclopride alone and the saline control due to inadequate error variance in statistical comparison.

Figure 4 presents the results for the fourth drug test, in which the effects of 0.5 mg/kg raclopride on amphetamine-induced behavioral alterations on DRL 10-s task were evaluated. As shown in the top panel, the IRT curves for four drug groups were similar and comparable to those described earlier. With respect to the quantitative analyses, as compared to the saline vehicle group, the group given amphetamine alone significantly decreased reinforced responses [ $F(1, 20) = 14.86$ ,  $P < 0.001$ ], increased non-reinforced responses [ $F(1, 20) = 6.683$ ,  $P < 0.05$ ], and decreased peak time [ $F(1, 20) = 27.821$ ,  $P < 0.001$ ]. The group given raclopride alone significantly decreased total responses [ $F(1, 20) = 22.093$ ,  $P < 0.001$ ], reinforced responses [ $F(1, 20) = 17.046$ ,  $P < 0.001$ ], non-reinforced responses [ $F(1, 20) = 9.076$ ,  $P < 0.01$ ], and peak rate [ $F(1, 20) = 31.586$ ,  $P < 0.001$ ]. The magnitudes of these decrements were lower for the group treated with a combination of raclopride and amphetamine. In this group, significant decreases were revealed for total responses [ $F(1, 20) = 12.147$ ,  $P < 0.01$ ], non-reinforced responses [ $F(1, 20) = 6.548$ ,  $P < 0.01$ ], and peak rate [ $F(1, 20) = 15.315$ ,  $P < 0.001$ ], but not for reinforced responses ( $P > 0.05$ ). Notice that no significant difference on burst response between the group treated with raclopride alone and the saline control due to inadequate error variance in statistical comparison.

## Discussion

The results of this experiment confirm that acute systemic 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. These gross changes in reinforced and non-reinforced responses correspondingly result in a horizontal leftward shift of the IRT distribution by amphetamine at the doses used. Based on quantitative analyses of the IRT data, amphetamine significantly decreases the peak time without any dramatic effect on the peak rate or burst response. Furthermore, this study demonstrated that these amphetamine-induced behavioral changes in the DRL task were dose-dependently and partially reversed by SCH23390 and raclopride. It is of particular interest to note that there was the identification of reversal effects on the amphetamine-induced leftward shift of the IRT distribution curve by dopamine receptor blockade.

In agreement with previous findings for research

on DRL task, the present treatment with amphetamine profoundly increased total responses and non-reinforced responses, but decreased non-reinforced responses. These results have been consistently observed for the operant behavior maintained on a DRL schedule of reinforcement with various interval criteria ranging from 10 sec up to 72 sec (1-3, 5, 7, 21, 24, 26, 27, 29, 34). The pattern of these response rates was altered by acute injection of amphetamine almost consistently over the four administrations in the SAL-AMP group. Thus, it is unlikely that this group given amphetamine alone had resulted in an induction of sensitization on the DRL responding. Although the present study was not designed to test psychostimulant induced behavioral sensitization after withdrawal following a specific drug administration regimen (13, 25), it is worth to noting that such a behavioral sensitization can be induced by a lower dose of amphetamine (0.5 mg/kg) in rats pretreated with six injections of 1.5 mg/kg amphetamine intermittently over 3.5 weeks as reported for a DRL 72-s behavior (1). In a recent study using a nose poke rather than the traditional lever press as the operant manipulandum, a sensitization-like outcome was reported for DRL 30-s behavior (23). In which, the rat was pretreated with five daily injections of 5 mg/kg of amphetamine and two days of withdrawal significantly increased the total number of nose pokes and decreased the response efficiency by increments for the IRTs of 1 sec, 28 sec, and 29 sec. Accordingly, the lack of sensitization-like effect observed with the present amphetamine-induced DRL behavioral changes over four sessions of drug treatment may be due to the lower dose used and the fewer number of injections applied here.

IRT analysis has been used to quantify two characteristics of DRL behavior, namely, behavioral inhibition and timing. The present quantitative analyses of the IRT data for amphetamine given alone are almost identical to what we have previously reported for DRL 10-s behavior (16). That is, amphetamine causes a horizontal leftward shift in the IRT distribution. Such a shifting effect was confirmed by a significant decrease in the peak time, with no dramatic changes to the peak rate or burst response. These IRT results for amphetamine treatment are in agreement with previous studies using quantitative analyses (1, 19, 24). Based on the lack of an effect on the burst response in the present study, it may be argued that the amphetamine treated rats have retained the ability to inhibit a certain degree for their early responding (IRTs of 1 sec and 2 sec). The peak time decrease caused by amphetamine has been suggested to be an underestimation of time possibly derived from the speeding up of the "internal clock" used for timing the experienced interval for reinforcement in the DRL task (16). Similarly, this argument can be used to support the non-reinforced



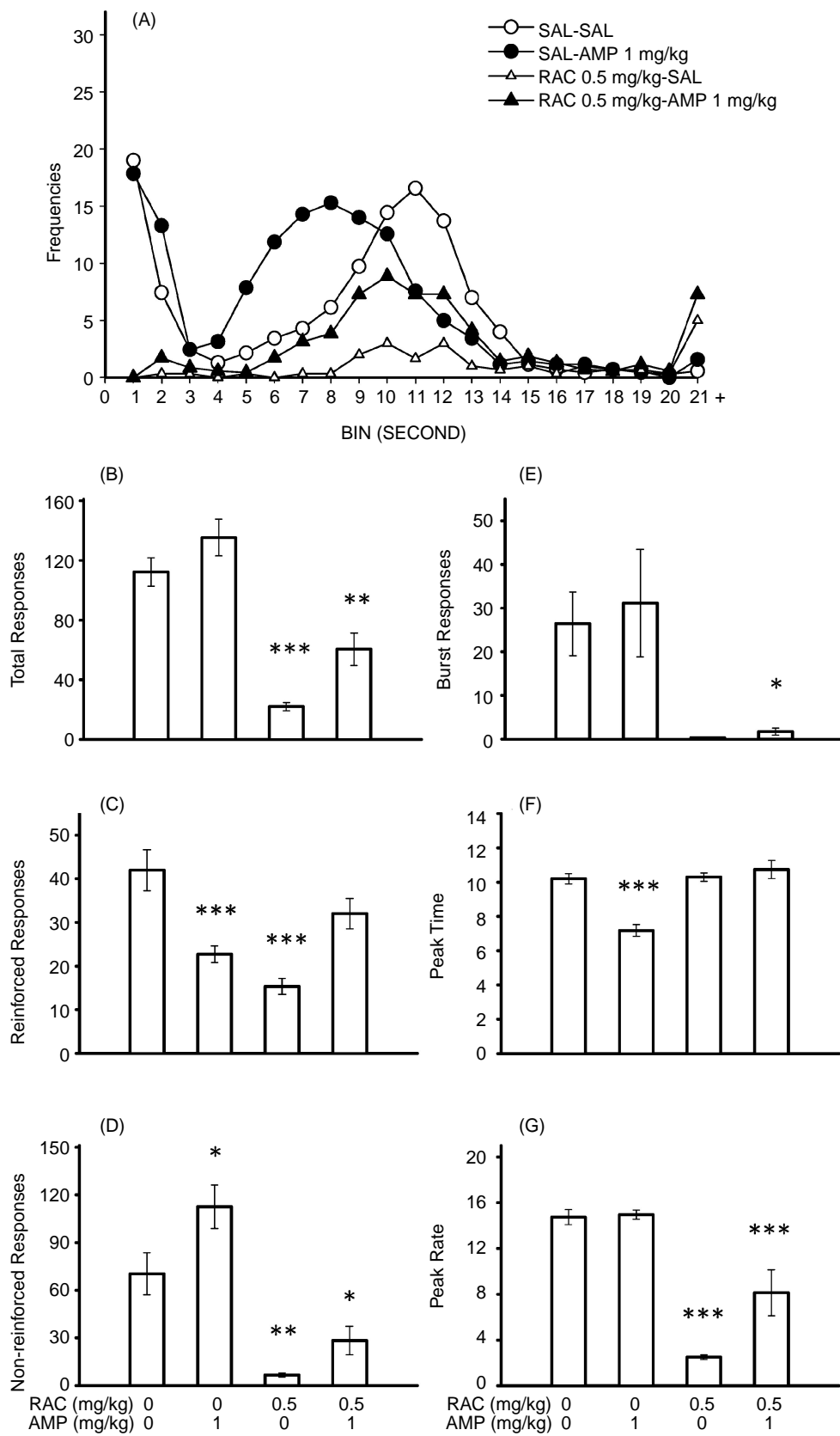


Fig. 4. Effects of 0.5 mg/kg raclopride on d-amphetamine-induced behavioral changes on the distribution of inter-response times (IRTs) and six relevant measures for a DRL 10-s task. Other details are as in Fig. 1.

responses increased by amphetamine. Notably, the IRTs at 6 sec, 7 sec, and 8 sec, which are just short to the required interval 10 sec for the present DRL task, were most profoundly increased as compared with other bins of the IRT rather than a broad increase among all other non-reinforced IRT bins. With no change in the peak rate under the present amphetamine treatment, it is unlikely that the leftward shift in IRT or the decrease in peak time was the result of drug-induced impairment of motor control. This is because the peak rate was not affected by amphetamine and remained at about the same level as the control. An intact bell-shaped IRT curve implies that the subjects can control the gross behavioral outputs for this timing related task. The predominant shortened timing effect of the amphetamine on temporal regulation observed in the present work is consistent with previous studies using a DRL task (19). Also, this observation is compatible and comparable to findings from studies using other time-based tasks such as peak interval (4, 14, 17) and temporal discrimination (6).

A major finding of the present study is that the amphetamine-induced response deficiency for DRL 10-s behavior was partially reversed by pretreatment with SCH23390 or raclopride. However, this reversal effect was manifested mainly at the lower doses of either dopamine antagonist tested. This is especially true for peak time, which indicates the time perception of the rats. SCH23390 at 0.02 mg/kg when given in combination with amphetamine was effective at reversing the peak time decrease caused by amphetamine and returning it to the level of saline control. Such a reversal effect was also generally true for the lower dose of raclopride (0.2 mg/kg), but to a lesser extent. The peak time for the group with a combined treatment of raclopride and amphetamine was still significantly lower than the control level ( $P < 0.05$ , see the panel F of Fig. 3). That was still statistically significantly less than that for amphetamine ( $P < 0.001$ ). Although the peak time for the group with combined treatment at the higher dose of SCH23390 or raclopride with amphetamine was close to that of the control level, it is difficult to verify this type of reversal effect for dopamine antagonism. The peak rate was more profoundly depressed by the higher dose of SCH23390 or raclopride given alone compared to the lower doses of these drugs. Nevertheless, the present findings demonstrate that the deficiency of DRL behavior induced by amphetamine can be reversed, if not completely, by pretreatment with SCH23390 or raclopride. To our knowledge, there has been no study to examine the effects of co-administration of amphetamine and dopamine receptor antagonist on DRL behavior. However, several lines of research in neuropsychopharmacology have shown similar results for a reversal effect of amphetamine-induced behavioral

changes by systemic injection of selective dopamine receptor antagonists, and these include prepulse inhibition task (33), conditioned place preference (15), a stimulus selection task called overshadowing (22), and self-stimulation on the lateral hypothalamus (12). Moreover, based on the findings that the rat's capability to discriminate amphetamine from saline was blocked primarily by a dopamine receptor antagonist rather than adrenergic and serotonergic antagonists (35), activation of dopamine receptors can be suggested as essential for amphetamine to function as a discrimination stimulus. Although the notion of dopaminergic antagonism can be supported by these results, it should be noted that whether the D1 or D2 receptor antagonists are effective to reverse amphetamine-induced behavioral alteration might be task dependent.

The behavioral deficiency and suppression induced by SCH23390 or raclopride given alone is consistent with a previous study from this laboratory using the same DRL 10-s task (16). The doses applied here were in the same range as those of previous work, in which SCH23390 was given at 0.05 and 0.1 mg/kg and raclopride was given at 0.2 and 0.4 mg/kg. As shown in Table 1, the injections at the higher dose of SCH23390 and both doses of raclopride dramatically suppressed operant responding that resulted in the missing data in the present study. The number of subjects that did not respond adequately on lever pressing was unexpectedly higher than previously. The explanation for this unexpected drug induced side effect is uncertain. However, one possibility is that these doses affect the motor system and this resulted in arrest of the operant response. Despite using a different type of schedule-controlled behavior (FR1), Fowler and Liou (10) reported that systemic injection of SCH23390 (0.01-0.16 mg/kg) and raclopride (0.05-0.8 mg/kg) dose dependently decreased operant responding. In addition, raclopride produced a so-called microcatalepsy as measured by an increase in the duration of the rat's muzzle entry into the reinforcer delivery well, while no such effect observed for SCH23390. Sensitization was observed for these behavioral impairments following repeatedly injection of both drugs. Even though behavioral deficiency and suppression induced by SCH23390 or raclopride given alone was observed in the present DRL task, the combination of dopamine receptor antagonist and amphetamine did reverse the behavioral changes induced by amphetamine itself.

The measures of the peak time and the peak rate of the IRT distribution curve for DRL responding deserve to be addressed due to the uniqueness of quantitative analysis of IRT data obtained from the present work. Such a quantitative method to analyze IRT data for DRL behavior has not been used over the

past decades. Until recently, a so-called peak deviation analysis was used to characterize changes in IRT distribution of DRL behavior induced by a variety of drugs including amphetamine (1, 24, 26). The peak deviation analysis quantifies IRT data into two measures, peak area and peak location. By a similar rational but with a slightly different emphasis, the present study used a simpler way to characterize the IRT curve in terms of peak time and peak rate. For amphetamine treatment, the decreases in peak area and peak location as measured by aforementioned peak deviation analysis are congruent with the decreased peak time observed in the present study. An advantage of using the present measure of peak time is that it allows the determination of the temporal regulation of DRL behavior as argued in previous work (28, 37). Accordingly, the decreased peak time caused by amphetamine in the present study could be conceivably interpreted as a result of the subject's underestimation of time (see above). It has been argued that this change in timing induced by amphetamine is relevant to brain dopamine systems in terms of neuropharmacology (20) and neuroanatomy (18). As such, the present findings whereby dopamine receptor antagonists reverse the decreased peak time of amphetamine is compatible and relevant to this notion addressed by Meck and his associates (20). In a more recent work by Cheung *et al.* (8), the effect of amphetamine on time differentiation on a free-operant psychophysical procedure was shown to be mediated by dopamine receptors, most critically by the D1 subtype. On the basis of these results, the temporal regulation involved in DRL responding would seem to be mediated by the brain dopamine systems. The shortened IRT responses produced by amphetamine may be primarily caused by an enhancement of dopamine release. This may be manifested in neurobehavioral and result in an attenuation or reversal of the dopamine receptor blockade. However, notwithstanding this explanation of the present findings for the reversal effects of the dopamine antagonists, the putative effects of general behavioral suppression induced by SCH23390 and raclopride suggests that caution is needed when interpreting our results.

In summary, the present study examined the effects of amphetamine given alone or in combination with SCH23390 or raclopride on DRL 10-sec behavior. The results showed that amphetamine alone produced behavioral deficiency on DRL responding, and this effect could be partially reversed by either SCH23390 or raclopride. Together, the current results suggest the involvement of the dopamine D1 and D2 receptors in the temporal regulation of the DRL response under psychostimulant drug treatment. However, which part of the brain dopamine systems mediate the effects

of amphetamine on the DRL response awaits for future investigation before a more solid conclusion can be drawn.

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