Differential Effects of Dopamine D_1 and D_2 Receptor Antagonists on Conditioned Orienting Behavior in the Rat

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Abstract

Brain dopamine (DA) systems are known to be important in regulation of behavior conditioned to appetitive stimuli. Nevertheless, despite a large body of evidence showing behavioral deficits in the operant conditioning paradigm produced by DA receptor blockade, there have been relatively few studies directly assessing behavioral changes in classical conditioning paradigm under this drug treatment. By employing an appetitive Pavlovian conditioning task, the present work investigated the effects of selective D_1 and D_2 receptor antagonists on the *expression* and *acquisition* of the conditioned orienting response (COR) and food-cup approach. SCH23390 (0, 0.05, and 0.10 mg/kg) and raclopride (0, 0.1, and 0.2 mg/kg) were administered via an intra-peritoneal route in a between-group design. Data from Experiment 1 showed that both SCH23390 and raclopride suppressed *expression* of the COR and food-cup approach, but only the impairment produced by raclopride reached a significant level. In Experiment 2, with SCH23390 being administered during the *acquisition* phase, the suppressed COR was completely restored in a subsequent (24 h later) drug-free session. In contrast, the suppressed COR in raclopride-pretreated groups was only partially restored. These findings support the view that the DA system plays a role in the neural substrates underlying this appetitive conditioning. In addition, D_2 receptors are more likely involved in the modulation of learning process of the COR than D_1 receptors.

Key Words: SCH23390, raclopride, appetitive Pavlovian conditioning, associative learning, acquisition, expression.

Introduction

Dopamine (DA) is thought to play an important role in mediating reward-related behaviors in both conditioned and reflexive domains (28). The evidence supporting this notion mainly relies upon findings of various types of operant conditioned behavior being suppressed by DA receptor blockade or lesions of the central DA pathway (see reviews by 4-6, 35-40, 43-45). Most of these previous studies focused on the mechanisms of DA underlying the performance of operant behavior and addressed on the motivational aspect of reinforcement (or reward) and motor performance. Based on the assumption regarding involvement of appetitive Pavlovian conditioning in operant behavior (3, 12), the impairment of operant performance by DA dysfunction might be the result of DA receptor blockade on the appetitive Pavlovian conditioning, especially during the acquisition phase of operant conditioning. A variety of behavioral responses can be used to measure conditioned responses in Pavlovian conditioning including the orienting response. To our knowledge, the effects of DA receptor blockade on appetitive Pavlovian conditioning measured by the orienting response associated with food reinforcer have not been reported. Thus, one purpose of this study was to examine the effects of systemic administration of DA receptor antagonists on this appetitive Pavlovian conditioning in the rat.

The specific conditioning procedure employed in the present study has been extensively used for

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revealing behavioral aspects of Pavlovian conditioning to a conditioned stimulus (CS) associated with the unconditioned stimulus (US) of food (see reviews by 22, 23). In the case of a light serving as the CS applied in this conditioning preparation, there are two types of conditioned responses which appear in rats: the conditioned orienting response (COR) and the food-cup approach. The former is CS-dependent, whereas the latter is US-dependent. In addition to their distinct characteristics from a behavioral perspective, the neural bases for COR and the food-cup approach are suggested to be independent (see reviews by 17, 18, 24). While the amygdaloid complex was implicated to be a key neural substrate for mediating the display of this food-motivated associative learning, the nigrostriatal DA system was highlighted to be involved in modulation of the expression of the COR (17, 25). A recent study examined how the neural connection between the amygdala and the striatum is involved in this type of condition responses by using an asymmetrical lesion model (20). The rat under reversible inactivation (provided by lidocaine) of the dorsolateral striatum contralateral to an excitotoxic lesion of the central nucleus of the amygdala failed to acquire the COR. Immediately following termination of the inactivation procedure, the COR significantly returned to the control level. Since the striatum is a major terminal area for brain DA systems, we then hypothesized that disruption of DA neurotransmission by blocking DA postsynaptic receptors should affect a subject's ability to display the COR. DA receptors have been classified into D_1 and D_2 receptor subtypes based on their ability to either increase or decrease the activity of enzyme adenylate cyclase (9, 41, 42). The DA receptor subtypes exhibit different properties in terms of their pharmacological profiles and mechanisms of action. The present study attempted to compare the effects of D_1- and D_2-selective receptor antagonists (SCH23390 and raclopride, respectively) on the COR. The dose range for each drug applied in the present study had a low potential to induce akinesia or catalepsy, as referenced to previous work using conditioned behavioral measurements from this (30-33) and other laboratories (2, 15, 16, 27, 46). Two experiments were designed to evaluate the dose effects of SCH23390 and raclopride on the *expression* and *acquisition* in the present conditioning task.

Materials and Methods

Subjects

Eighty male Wistar rats, which weighed 300-325 g at the beginning of the experiment, were housed individually in a vivarium with a 12-h light/dark cycle (lights on 07:00-19:00). The temperature of the vivarium was maintained at 23±1°C. All rats were purchased from the Experimental Animal Center at National Taiwan University Hospital, Taipei, Taiwan. They were allowed to adjust to their home cage environment, with access to food and water ad libitum for 10 days following arrival. Subsequently, a food deprivation regimen was conducted by gradually reducing the daily allowable amount of food. Thereafter, each rat was fed approximately 18 g of laboratory chow in its home cage no sooner than 30 min after the end of each daily experimental session. The subjects were thus maintained at 85% of their free-feeding body weights throughout the experiment. Tap water was continuously available in each home cage. Training and/or test sessions were administered at the same time period during the lighted portion of the day. Treatment of rats complied in all respects with the Chinese Psychological Association's ethical standards for the use of animals in research (8).

Drugs

SCH23390 hydrochloride (Tocris Cookson, Bristol, UK) and raclopride l-tartrate (Sigma, St. Louis, MO, USA) were separately dissolved in 0.9% physiological saline. In all cases, the doses used for SCH23390 were 0.05 and 0.1 mg/kg, while those used for raclopride were 0.1 and 0.2 mg/kg. Injections of the drug and vehicle were administered intraperitoneally (IP) at a constant volume of 1 ml/kg of body weight, 1 h before the commencement of a behavioral session.

Apparatus

The apparatus consisted of four individual chambers placed in a sound-attenuated room with dim light illumination. The inside diameters were 20 (H) × 22 (W) × 28 (L) cm for each chamber. Aluminum panels formed the front and back walls, and darkened Plexiglas comprised the remaining sides and top. Stainless steel bars (with diameters of 5 mm) were set 11 mm apart to provide flooring. The cup that served as the food dispenser was located in the center of the front panel, 4 cm above the floor. The visual stimulus during experimental sessions was provided by a light bulb (2.5 W) that was positioned in the ceiling panel. White noise was provided during all experimental sessions.

Procedures

Two experiments were carried out separately with different groups of rats. Experiment 1 evaluated the effects of vehicle, two doses of SCH23390, and two doses of raclopride on *expression* of the appetitive

conditioning behavior; while Experiment 2 evaluated the effects of the above treatments on *acquisition* of the conditioned behavior. Each dose or vehicle treatment was conducted on a group of eight rats (n=8). Therefore, there were ten groups in total for the present study. The experiment was conducted in three successive phases: 1) contextual exploration for one session, 2) pre-conditioning habituation for two sessions, and 3) light-food pairing for six (or seven) sessions.

In terms of conditioned orienting behavior, the training/testing procedures used in the present study were modified from those of Gallagher and associates (19, 20). Food-deprived subjected were first trained to access food pellets (each weighing approximately 10 mg) from the food cup for one session. Each subject was initially allowed to explore the chamber for 5 min. In the following 20 min, food pellets were delivered in a variable-interval (VI) schedule of 2 min. At the end of this session, none of the subjects failed to perform the food-cup approach response. For the next two sessions, the subject was initially placed in the chamber for 5 min and then exposed to 20 lights-on trials. The light bulb was continuously illuminated for 10 s for each light-on trial, with an inter-trial interval of 2 min on average. There was no food delivery during these two pre-conditioning sessions. The conditioning phase was subsequently begun on the fourth day. The procedures for the conditioning phase were similar to those applied in the pre-conditioning habituation phase. However, during conditioning, a food pellet (~ 10 mg for each contingency) was immediately delivered into the food cup at the time the light was turned off. In Experiment 1, drug or vehicle treatment was administered on the sixth and seventh days. Subjects were randomly divided into five groups each receiving a particular dose treatment of a drug or vehicle. In order to prevent any confounding effect resulting from the injection procedure, all subjects in this experiment were injected with vehicle control of saline on the sixth day. In Experiment 2 concerning the drug effects on the *acquisition* of this behavior, each subject received a specific dose treatment by injection repeatedly conducted on the first to the fifth days of the conditioning phase. Twenty-hours later, a behavioral test was conducted under a drug-free state as the sixth session of this experiment.

Behavioral data were collected from the pre-conditioning and conditioning sessions. Two observers, who did not know the experimental design, judged the appearance of the COR and food-cup approach for each lights-on trial. During this 10-s period, a COR was counted if the subject stood on its hind limbs with both forelimbs off the floor (but with no grooming). Rapid horizontal movement towards the food cup defined as the food-cup approach. Since there was no food pellet presented in the pre-conditioning habituation phase, only the COR was measured. Consistently with previous studies (19, 20, 22), the COR primarily appeared in the first 5-s interval of the 10-s light-on trial, while the food-cup approach mostly occurred in the latter 5-s interval. Scores from the two independent observers were very consistent as revealed by a high inter-rater correlation coefficient (r=0.95). Therefore, the data used for statistical analyses were based the averaged scores from the two observers.

Statistical Analysis

Statistical analyses were conducted using commercialized software (Statistica version 5.5, Statsoft, Tulsa, OK, USA). The drug effects were assessed with a mixed two-way analysis of variance (ANOVA), with the dose as the between-subject factor and the experimental session as the within-subject factor. If a significant interaction was detected, the Newman-Keuls test was used for *post hoc* comparisons. A significance level was set at $P<0.05$ for all tests. All means are presented along with the standard errors.

Results

In order to analyze data in a dose-related fashion specifically for each drug, the same vehicle control group served repeatedly as the control for the two drugs in each experiment. Accordingly, the vehicle data presented in Figs. 1 and 2 are from an identical group, so are the cases for Figs. 3 and 4.

During the pre-conditioning habituation phase, the spontaneous orienting response to the visual stimulus (light-only) was apparent on the first day, but was significantly diminished on the second day. The averaged responses (with standard errors) for all three groups with subsequent treatment of vehicle and two doses of SCH23390 to the visual cue on the first and second days of presentation in Experiment 1 were 15.5±0.9 and 5.9±1.3, respectively. Corresponding responses for raclopride in Experiment 1 were 15.3±0.9 and 6.5±1.0; those for SCH23390 in Experiment 2 were 15.5±0.8 and 8.5±0.6; and those for raclopride in Experiment 2 were 15.4±0.9 and 10.3±1.1, respectively. This phenomenon of a subject's habituation to the light-only presentation was confirmed by two-way ANOVA (3 groups by 2 days) conducted on the orienting response for each experiment. All four ANOVAs consistently yielded a significant day effect (F value range 41.4-191.6, P <0.0001). Neither the group main effect nor the group-by-day interaction was significant (P>0.05).

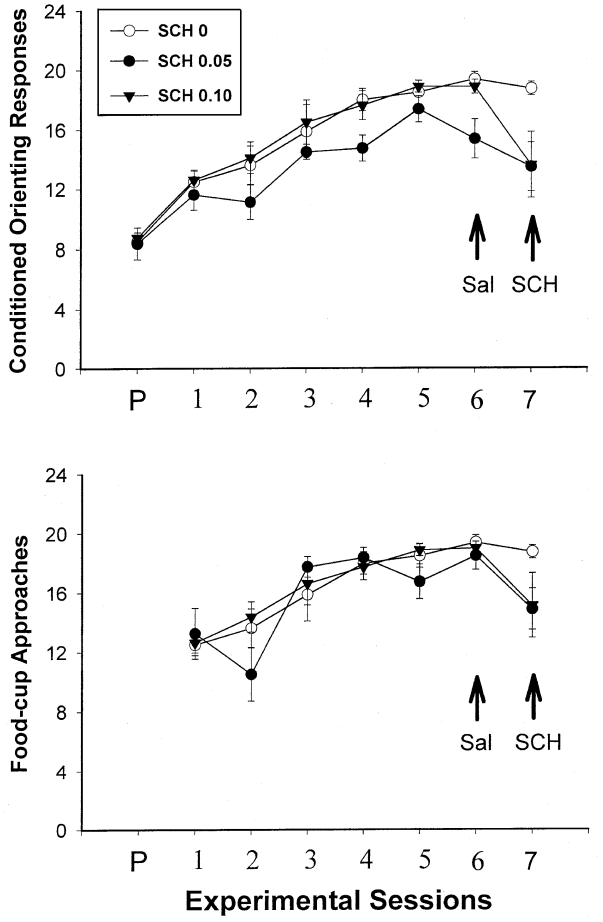

Fig. 1. Dose effects of SCH23390 on the *expression* of conditioned orienting behavior in Experiment 1. The conditioned orienting response (top) and the food-cup approach (bottom) are presented for seven sessions in which light, serving as the conditioned stimulus, was paired with food as the unconditioned stimulus. P represents the spontaneous orienting responses to the visual cue (light-only) presentation on the second day of the pre-conditioning habituation phase. Three groups (n=8 each) all received saline (Sal) on the sixth day, but received SCH23390 (SCH) at doses of 0, 0.05, and 0.10 mg/kg, respectively, on the seventh day. Each data point denotes the mean±1 s.e.m.

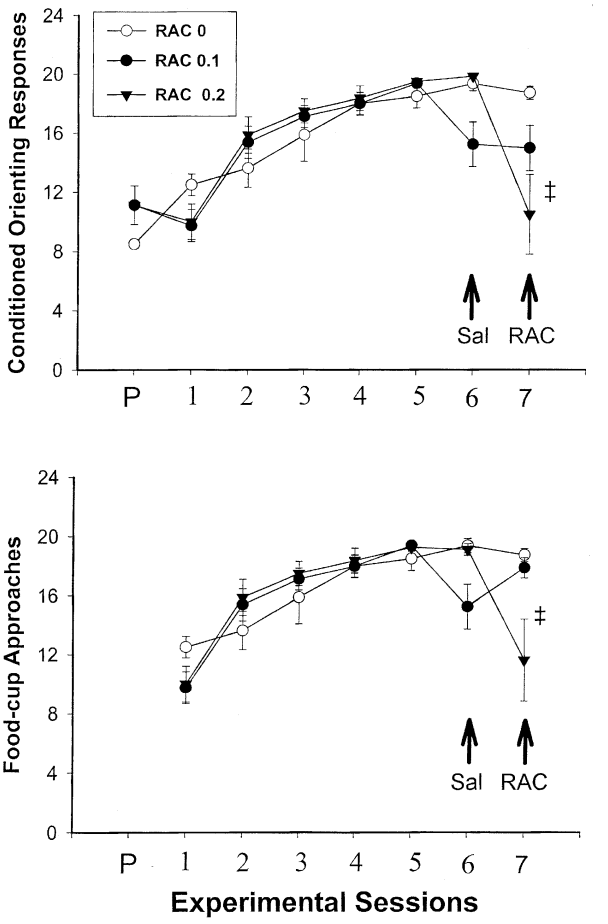

Fig. 2. Dose effects of raclopride on the *expression* of conditioned orienting behavior in Experiment 1. The conditioned orienting response (top) and the food-cup approach (bottom) are presented for seven sessions in which light, serving as the conditioned stimulus, was paired with food as the unconditioned stimulus. P represents the spontaneous orienting responses to the visual cue (light-only) presentation on the second day of the pre-conditioning habituation phase. Three groups (n=8 each) all received saline (Sal) on the sixth day, but received raclopride (RAC) at doses of 0, 0.1, and 0.2 mg/kg, respectively, on the seventh day. Each data point denotes the mean±1 s.e.m. (++ P<0.01 as compared with the control group from the indicated session by a 3×2 ANOVA)

The dose effects of SCH23390 on *expression* of the present conditioned behavior are illustrated in Fig. 1. As shown in the top panel of Fig. 1, none of the spontaneous orienting responses of the three groups differed to the visual cue (light-only) presentation on the second day of the pre-conditioning habituation phase. One-way ANOVA confirmed this observation by yielding a non-significant between-group effect (P>0.05; see session P in the top panel of Fig. 1). Subjects of all three groups consistently acquired the COR over five conditioning sessions. The results of two-way ANOVA (3 groups by 5 sessions) revealed a significant session main effect (F(4, 84)=23.2, P< 0.01). Neither the group main effect nor the group-by-session interaction was significant. The dose effects of SCH23390 on expression of the COR were evaluated by two-way ANOVA (3 groups by 2 days); data for the sixth and seventh sessions are presented in the top panel of Fig. 1. The results of ANOVA revealed significant main effects of group and session factors (F(2, 21)=7.04 and F(1.21)=5.44, respectively; both P<0.01). The test of the group-by-session interaction did not reach a significant level. The apparent difference between SCH23390-treated groups and the vehicle control group for the seventh session fell short of statistical significance as revealed by a separate one-way ANOVA (F(2, 21)=3.4, P= 0.051).

As shown in the bottom panel of Fig. 1, subjects

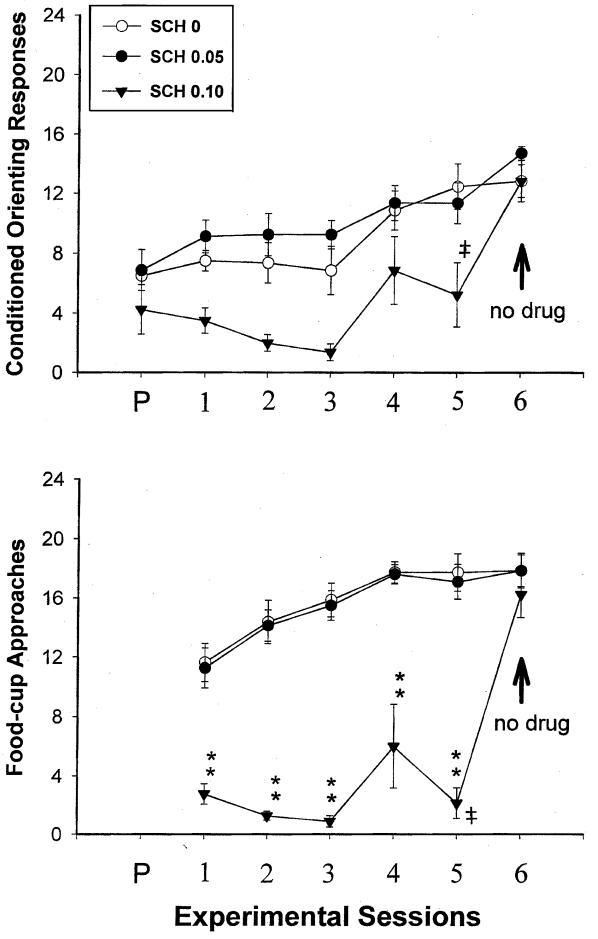

Fig. 3. Dose effects of SCH23390 on the *acquisition* of conditioned orienting behavior in Experiment 2. The conditioned orienting response (top) and the food-cup approach (bottom) are presented for six sessions in which light, serving as the conditioned stimulus, was paired with food as the unconditioned stimulus. P represents the spontaneous orienting responses to the visual cue (light-only) presentation on the second day of the pre-conditioning habituation phase. Each group (n=8) received a specific dose of SCH23390 (0, 0.05, or 0.10 mg/kg) from sessions 1 to 5. A drug-free test was conducted in the sixth session. Each data point denotes the mean±1 s.e.m. Any values with an asterisk or cross sign in the SCH23390 (0.10 mg/kg)-treated group significantly differed from corresponding values in the vehicle control group (** P<0.01 by a 3×5 ANOVA; ++ P<0.01 by a 3×2 ANOVA)

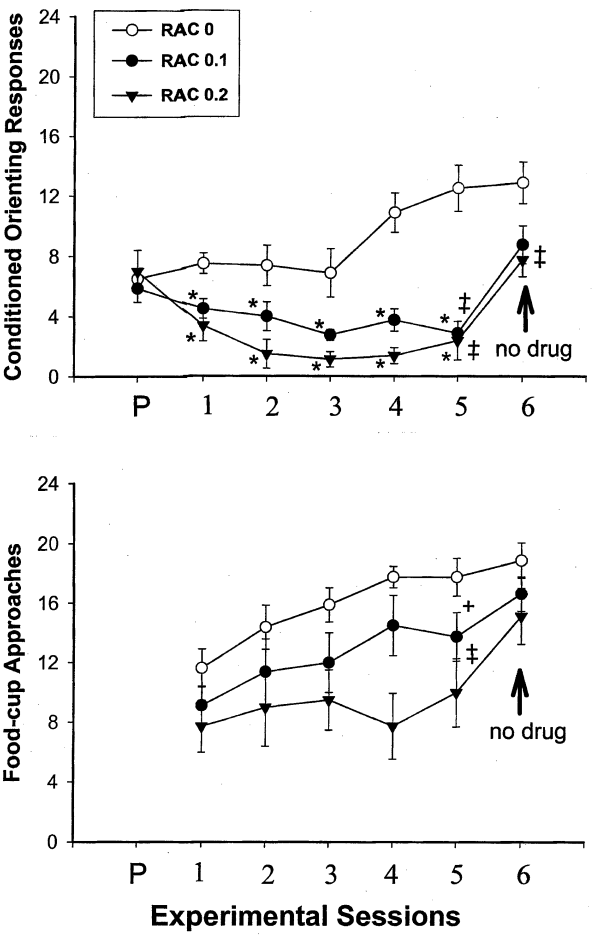

Fig. 4. Dose effects of raclopride on the *acquisition* of conditioned orienting behavior in Experiment 2. The conditioned orienting response (top) and the food-cup approach (bottom) are presented for six sessions in which light, serving as the conditioned stimulus, was paired with food as the unconditioned stimulus. P represents the spontaneous orienting responses to the visual cue (light-only) presentation on the second day of the pre-conditioning habituation phase. Each group (n=8) received a specific dose of raclopride (0, 0.1, or 0.2 mg/kg) from sessions 1 to 5. A drug-free test was conducted in the sixth session. Each data point denotes the mean±1 s.e.m. (* P<0.05, as compared with the control group for the indicated session from a 3×5 ANOVA; + P<0.05, ++ P< 0.01 as compared with the control group for the indicated session from a 3×2 ANOVA)

of all three groups reliably performed the food-cup approach over the first five sessions. The results of two-way ANOVA (3 groups by 5 sessions) only revealed a significant session effect (F(4, 84)=21.93, P<0.01). Regarding the food-cup approach in the sixth and seventh sessions, results of two-way ANOVA only revealed a significant session effect (F(1, 21) =10.08, P<0.01). A separate one-way ANOVA did not yield a significant between-group difference for the data regarding the food-cup approach in the seventh session.

The dose effects of raclopride on *expression* of the present conditioned behavior are shown in Fig. 2. Prior to the conditioning session, the habituated spontaneous orienting responses to the visual cue (light-only) presentation did not significantly differ among the three groups as revealed by one-way ANOVA (P>0.05; see session P in the top panel of Fig. 2). Subjects of all three groups consistently acquired the COR over five conditioning sessions. The results of a 3 by 5 ANOVA only revealed a significant session main effect (F(4, 84)=38.9, P<

0.01). The dose effects of raclopride on *expression* of the COR were evaluated by a 3 by 2 ANOVA, for which data of the sixth and seventh sessions are presented in the top panel in Fig. 2. The results of ANOVA revealed significant main effects of group and session factors ($F(2, 21)=5.7$ and $F(1.21)=7.36$, respectively; both $P<0.05$). The test of the group-by-session interaction also reached a significant level ($F(2, 21)=5.6$, $P<0.05$). *Post hoc* comparisons indicated that the group treated with the higher dose (0.2 mg/kg) of raclopride expressed significantly fewer COR compared to the control group on the drug-testing day ($P<0.01$). Such a case was also true when comparing the change from the sixth to the seventh day.

As shown in the bottom panel of Fig. 2, subjects of all three groups reliably performed the food-cup approach over the first five sessions. The results of a 3 by 5 ANOVA only revealed a significant session effect ($F(4, 84)=37.86$, $P<0.01$). Regarding the food-cup approach of the sixth and seventh sessions, results of a 3 by 2 ANOVA revealed a significant group effect ($F(2, 21)=3.6$, $P<0.05$) and a significant group-by-session interaction ($F(2, 21)=7.58$, $P<0.01$). As revealed by *Post hoc* comparisons, the group treated with the higher dose (0.2 mg/kg) of raclopride expressed significantly fewer food-cup approaches compared to the control group on the drug-testing day ($P<0.01$). Such a case was also true when comparing the change from the sixth to the seventh day.

Figure 3 depicts the dose effects of SCH23390 on *acquisition* of the present conditioned behavior. The spontaneous orienting responses to the light-only stimulus between groups were not significant before the conditioning procedure was applied ($P>0.05$; see session P in the top panel of Fig. 3). During the 5-day conditioning phase with drug treatment, a 3 by 5 ANOVA revealed significant main effects of group and session factors ($F(2, 21)=14.06$ and $F(4, 84)= 8.54$, respectively; both $P<0.01$). The test of the group-by-session interaction did not reach statistical significance. Comparing the last (fifth) day of drug administration and the sixth day without an injection, a 3 by 2 ANOVA yielded a significant group effect ($F(2, 21)=3.59$, $P<0.05$), a significant session effect ($F(1, 21)=15.42$, $P<0.01$), and a significant interaction ($F(2, 21)=4.74$, $P<0.05$). *Post hoc* comparisons indicated that the group treated with the higher dose (0.10 mg/kg) of SCH23390 produced significantly fewer COR compared to the control group on the fifth day ($P<0.01$). However, no significant between-group difference of COR was revealed when subjects were free of injection on the sixth day.

The dose effects of SCH23390 injected during five conditioning sessions on food-cup approach are presented in the bottom panel of Fig. 3. A 3 by 5 ANOVA yielded a significant group effect ($F(2, 21 =76.18$, $P<0.01$), a significant session effect ($F(4, 84) =12.11$, $P<0.01$), and a significant interaction ($F(8, 84)=2.28$, $P<0.05$). *Post hoc* comparisons indicated that the group treated with the higher dose (0.10 mg/kg) of SCH23390 produced significantly fewer food-cup approaches compared to the control group on each of these 5 days (all $P<0.01$). Comparing data of the fifth and sixth sessions, a 3 by 2 ANOVA yielded a significant group effect ($F(2, 21)=20.67$), a significant session effect ($F(1, 21)=50.14$), and a significant interaction ($F(2, 21)=41.8$) (all $P<0.01$). *Post hoc* comparisons indicated that the group treated with the higher dose (0.10 mg/kg) of SCH23390 produced significantly fewer food-cup approaches compared to the control group on the fifth day ($P< 0.01$). But, no significant between-group difference of the food-cup approach was revealed when subjects were free of injection on the sixth day.

Figure 4 displays the dose effects of raclopride on *acquisition* of the present conditioned behavior. Similar to those reported above, the spontaneous orienting responses to the light-only stimulus between groups were not significant before the conditioning procedure was applied ($P>0.05$; see session P in the top panel of Fig. 4). For data of sessions 1 to 5 as shown in the top panel of Fig. 4, all three tests from a 3 by 5 ANOVA reached a significant level at $P<0.01$ ($F(2, 21)=25.19$ for the group effect, $F(4, 84)=4.19$ for the session effect, and $F(8, 84)=4.31$ for the interaction). *Post hoc* comparisons indicated that the group treated with either dose of raclopride produced significantly fewer COR compared to the control group over each of the five sessions ($P<0.05$). Comparing the last (fifth) day of drug administration and the sixth day without an injection, a 3 by 2 ANOVA yielded a significant group effect ($F(2, 21) =13.6$), a significant session effect ($F(1, 21)= 59.83$), and a significant interaction ($F(2, 21)= 12.08$) (all $P <0.01$). Although the raclopride-treated groups produced fewer COR in the sixth session when no injection given compared to those in the fifth session on the last day of drug administration, the curves of these two groups had not fully returned to the control level on the sixth day. *Post hoc* comparisons indicated that the group treated with either dose of raclopride produced significantly fewer COR compared to the control group in both the fifth and sixth sessions (all $P<0.01$).

The dose effects of raclopride injected during five conditioning sessions on food-cup approaches are presented in the bottom panel of Fig. 4. Regarding the data of the first five sessions, a 3 by 5 ANOVA yielded significant effects on testing the group factor ($F(2, 21)=5.05$, $P<0.05$) and the session factor ($F(4, 84)=7.26$, $P<0.01$); but not the interaction.

Comparing data in the fifth and sixth sessions, a 3 by 2 ANOVA revealed a significant session effect ($F(1, 21)=13.17$) and a significant group-by-session interaction ($F(2, 21)=3.75$) (both $P<0.05$). *Post hoc* comparisons indicated that both groups treated with raclopride produced significantly fewer food-cup approaches compared to the control group in the fifth sessions ($P<0.05$ for the 0.1 mg/kg group and $P< 0.01$ for the 0.2 mg/kg group). No significant between-group differences were confirmed in the sixth session.

Discussion

The present study examined the relative roles of D_1 versus D_2 DA receptor subtypes in appetitive Pavlovian conditioning. SCH23390 and raclopride, administered peripherally, were used to determine whether the effects of the compounds on appetitive conditioned responses were attributable to blockade of D_1 or D_2 DA receptors. Although data from Experiment 1 show that both SCH23390 and raclopride appeared to suppress the *expression* of COR, only the impairment produced by raclopride reached a significant level. The patterns of impairment of the *expression* of the food-cup approach produced by both drugs were similar to those described for the COR. In Experiment 2, with SCH23390 administered during the *acquisition* phase, the suppressed COR was completely restored in a subsequent (24 h later) drug-free session. In contrast, the suppressed COR in raclopride-pretreated groups was only partially restored.

Regarding the specific conditioning preparation employed in the present study, results of both the COR and the food-cup approach of control subjects were in agreement with previous reports (19, 20). All groups initially oriented to the light at relatively high levels on the first pre-conditioning day. These spontaneous orienting responses were significantly habituated on the next day. During the conditioning phase, the COR could apparently be established over five sessions in subjects with no drug injection (or those vehicle control groups). Such a case was also true for the food-cup approach. The association capability for determining the CS-US pairing in the appetitive Pavlovian conditioning can be a basic process for various types of response generation in a behavioral system, whose operation requires an intact functioning sensorimotor circuit.

Raclopride, but not SCH23390, significantly impaired the expression of conditioned responses including both the COR and food-cup approach. These results suggest that DA D_2, but not D_1, receptors are involved in the display of the conditioned response of appetitive Pavlovian conditioning. It is unlikely that the failure of SCH23390 to produce such impairment was due to inadequate doses applied in the present work, since it is generally true that SCH23390 is more potent than raclopride in disturbing behavioral performance on either conditioned or reflexive tasks with regard to simultaneous comparisons of these two drugs in a single study (i.e., 2, 15, 16, 33). Also, as reflected from the dose of 0.1 mg/kg used for each drug in Experiment 2, SCH23390 produced a higher magnitude of suppression of the food-cup approach than did raclopride. Thus, the differential effects of SCH23390 and raclopride on preventing the expression of the conditioned response of appetitive Pavlovian conditioning are attributed to distinct drug reactions from comparable doses.

One might argue that raclopride simultaneously impairing the expression of the COR and food-cup approach can be attributed to drug-induced nonspecific effects (i.e., motor difficulty), based on postulation of the independence of the present two conditioned responses. It should be noted that none of the subjects showed any akinetic or cataleptic reactions under drug treatment according to the experimenter's observations, yet this argument does not necessarily mean that a general motor deficit induced by the drugs is completely excluded. Thus, decreases in the conditioned responses of this part of data may be further attributed to a variety of factors other than non-specific motor deficit induced by raclopride. Scrutinizing the data of Experiment 2 may yield a more-reasonable inference to address this issue. Since subjects in Experiment 2 only received drug treatments during the *acquisition* phase, but not on the last drug-free test day, those nonspecific effects were unlikely to appear on the test day which was 24 h after drug treatment. While SCH23390-pretreated subjects resumed elicitation of both conditioned responses of the COR and the food-cup approach on the drug-free test day, such recovery was not the case for raclopride-pretreated subjects. In regard to these data for raclopride shown in Fig. 4, pre-exposure to the raclopride treatment significantly suppressed the COR in the drug-free test. In contrast to this suppressive effect on the COR, the food-cup approach was not significantly affected by pre-exposure to the raclopride treatment. Hence the current results are not likely the outcomes of nonspecific actions of drug administration. In contrast to pre-exposure to the raclopride treatment, behavioral suppression did not occur with SCH23390 pre-exposure. Furthermore, despite the behavioral performance generally being impaired by DA receptor blockade, a certain part of the behavioral components remained invulnerable when a selective DA receptor antagonist was administered. Another point concerning the latent learning is worth noting (29). The subjects showed no apparent conditioned responses under treatments

administered over five conditioning sessions, but performed the COR and food-cup approach on the subsequent drug-free test session. This restoration of conditioned responses was more profound for SCH23390 pre-treated subjects than raclopride pre-treated ones. Thus, it is likely that the subjects acquire the stimulus-response association in the manner of latent learning.

The present findings for raclopride support the hypothesis that display of the COR is impaired by DA receptor blockade (D_2 subtype in this case). The effects of raclopride on the COR were positive when this drug was administered in either the *acquisition* or the *expression* phase. These data indicate that D_2 receptors play a pivotal role in both the *acquisition* and *expression* of the COR. As a critically important terminal area of brain DA systems, the striatum is implicated in learning this type of behavior. Using a functional disconnection lesion procedure, Han and associates (20) reported that the *expression* rather than the *acquisition* of the COR depends on the activation of the dorsolateral striatum connecting to the central nucleus of the amygdala. Their data showed that the COR returned to the control level once the striatal inactivation procedure was terminated. Similarly, the present study found that the suppressed COR by SCH23390 during the acquisition phase rebounded to the control level by the last drug-free test day. Therefore, given that brain DA is involved in the COR, DA D_1 and D_2 receptor subtypes located in different areas may play differential roles in the acquisition and maintenance of the COR. For instance, further work to determine the effects of DA antagonist(s) infused into discrete striatal subareas or distinct DA terminal areas on this conditioning task is necessary before this notion can be confirmed.

Based on the implication that the COR represents the output of attention-related processes (14, 17-19, 22-25), the inference based on the current data regarding the involvement of DA in the attentional processing is likely to be true. Although the assumption that DA systems are directly involved in attentional processing is still being debated (10), there is a large body of evidence highlighting the role of DA in attentional processes that require execution control. For example, dopamine systems are reputed to be essential for a rat to correctly respond to choice reaction time procedures, which is an analogue of the human performance test of attention (7, 21, 34). While these data were collected from animal studies, they are also consistent with results from a human study using the same task (11). From another human study, it was argued that the DA D_2 receptor is important in the modulation of selective attention on the basis of observing the suppression of a specific transient electric response to the attendant stimuli by haloperidol (1). Therefore, COR suppression produced by raclopride implies that DA D_2 receptors may be involved in the attentional process during appetitive Pavlovian conditioning, given that the COR results from goal-directed attentional processes (17, 22, 23, 25). However, it should be noted that further work with manipulation of conditioned stimulus paired with and without food (i.e. CS+ vs. CS-) is needed for directly addressing this attention-related issue.

It is interesting to compare the present data with recent findings from others regarding the relative roles of D_1 and D_2 receptor subtypes in appetitive Pavlovian conditioning. Determined by the elevation of locomotor activity in hungry rats exposed to a CS (a light in this case) before food delivery, pimozide as a nonselective DA receptor blocker produced an overall (35-min) suppression of locomotor activity, but left relatively increased locomotion from the pre- to post-CS (5-min) period intact (26). These results imply that the incentive motivational properties of the CS are invulnerable to DA receptor blockade. Using a similar paradigm for minimizing the motor requirement, Horvitz and Eyny (27) trained rats to enter a food trough during pellet delivery that was signaled by a tone on a variable-time 70-s schedule for 16 days. The dose effects of raclopride (0, 0.2, and 0.4 mg/kg) on the *expression* of this type of conditioned behavior were determined the following day. Head entries into the food compartment in the pre-CS period were significantly decreased by raclopride, but those measured during the post-CS period remained unaffected by drug treatment. It is of particular interest, from our viewpoint, in comparing the drug effects on the *acquisition* and *expression* phases of this appetitive-conditioned behavior. Most recently, Eyny and Horvitz (13) examined the conditioning response of head entry of rats in a drug-free test session 24 h after one session of tone-food conditioning (in 28 paired trials) under selective DA receptor blockade. Head entries as the conditioned response to the tone decreased in rats that had been treated with SCH23390 (0.04, 0.08, and 0.16 mg/kg), whereas they increased in rats treated with raclopride (0.2, 0.4, and 0.8 mg/kg). Together, despite the difficulties of a cross-study comparison due to discrepancies in several detailed procedures of the conditioning preparation, the present results and previous findings mentioned above support the notion that the differential roles of D_1 and D_2 subtype receptors in the appetitive conditioning can be consistently addressed.

In conclusion, our findings support the view that the DA system plays a role in the neural substrates underlying the orienting response associated with food in appetitive Pavlovian conditioning. In addition,

D_2 receptors are more likely involved in the modulation of learning process of the COR than D_1 receptors.

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