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Role of D_1 and D_2 dopamine receptors in the acquisition and expression of flavor-preference conditioning in sham-feeding rats

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Abstract

The present study examined the effects of D_1 and D_2 antagonists on flavor-preference conditioning by the sweet taste of sucrose. All sessions were conducted under sham-feeding conditions to minimize post-ingestive influences. The rats were trained in alternating, one-bottle sessions to sham-feed a 16% sucrose solution containing one novel flavor (CS+) and a less-preferred 0.2% saccharin solution containing a different flavor (CS−). Three groups of food-restricted rats were treated with either vehicle (control group), the D_1 antagonist, SCH23390 (200 nmol/kg), or the D_2 antagonist, raclopride (200 nmol/kg) during one-bottle training. A fourth group (yoked group) was vehicle-treated and its training intakes were matched to that of the D_1 and D_2 drug groups. Preferences were assessed in two-bottle tests with the CS+ and CS− flavors presented in mixed 8% sucrose + 0.1% saccharin solutions following systemic doses of 0, 200, or 800 nmol/kg of either the D_1 or D_2 antagonists. All groups significantly preferred the CS+ flavor in vehicle tests, although the preferences were weaker in the D_1, D_2, and yoked groups compared to the control group. All groups selectively reduced their CS+ intakes when treated with either D_1 or D_2 antagonists during two-bottle testing, and the CS+ preference was blocked at the higher doses. These data show that D_1 and D_2 receptor antagonists block the expression of a sucrose-conditioned preference, but produces substantially lesser effects upon the acquisition of this form of flavor conditioning.

Keywords: Conditioned flavor preference; Sham-feeding preparation; Dopamine; D_1 receptor; D_2 receptor; SCH23390; Raclopride; Expression studies; Acquisition studies

1. Introduction

Food preferences are based, in part, on learned associations between the various flavor elements in foods, referred to as flavor–flavor conditioning, and between the food's flavor and its post-ingestive nutritive consequences, referred to as flavor–nutrient conditioning [24]. In an early example of flavor–flavor learning, rats were trained with one arbitrary flavor (the conditioned stimulus or CS+) mixed into a concentrated saccharin solution, and a second flavor (the CS−) mixed into a dilute saccharin solution [12]. In a subsequent choice test, the rats preferred the CS+ to the CS− flavor when both were presented in solutions containing the same amount of saccharin. In a common paradigm used to study flavor–nutrient learning, rats are trained with one flavored solution (the CS+) paired with an intragastric nutrient infusion (the unconditioned stimulus or US), and a second flavored solution (the CS−) paired with a water infusion. With many, but not all types of nutrients, the rats display a strong CS+ preference in subsequent choice tests [24]. Different neural processes may mediate these two types of flavor learning because flavor–nutrient conditioning is possible with delays between the CS and US of several minutes or more, whereas the US flavor must be closely associated with the CS flavor for flavor–flavor conditioning to occur [8,12,18].

In parallel studies, our laboratories have been investigating the pharmacological mechanisms involved in flavor–flavor [31,32] and flavor–nutrient [1] conditioning. To

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study flavor–flavor learning, the subject of the present report, we have adopted the sham-feeding procedure in which ingested solutions drain out an open gastric fistula thus minimizing the post-ingestive effects of the solutions [29]. An advantage of this procedure is that nutritive as well as nonnutritive solutions can be used as unconditioned stimuli to produce flavor–flavor learning in the absence of post-ingestive nutritive conditioning. Further, rats consume substantial amounts of sapid solutions during sham-feeding sessions so that their exposure to the conditioning stimuli during one-bottle training sessions is maximized. In addition, their elevated intakes during two-bottle sham-feeding tests provide a high baseline to evaluate drug effects on flavor preferences.

Using the sham-feeding technique, Yu et al. [31,32] trained rats to drink distinctively flavored (e.g., grape and cherry) 16% sucrose and 0.2% saccharin solutions during one-bottle sham-feeding trials. In subsequent two-bottle tests, the rats preferred the sucrose-paired flavor (CS+) to the saccharin-paired flavor (CS−) when both were presented in mixed sucrose–saccharin solutions. This flavor preference was attributed to the reinforcing effect of the sucrose taste since the sugar's post-ingestive actions were minimized by the open gastric fistula. Note that while saccharin and sucrose are both sweet, rats prefer concentrated sucrose solutions to saccharin solutions in two-bottle choice tests [6,16] and sham-feed substantially more sucrose than saccharin in one-bottle tests [26]. Thus, the taste of sucrose is a more potent US than the taste of saccharin for flavor–flavor learning.

Yu et al. [31] found that the general opioid antagonist, naltrexone, significantly reduced the sham intakes of the sucrose and saccharin solutions in sham-feeding rats, but yet had little or no effect on the acquisition or expression of the flavor preferences conditioned by the sweet taste of sucrose. In contrast, Yu et al. [32] observed that selective antagonists of either D_1 (SCH23390) [5,14,27] or D_2 (raclopride) [15,19] receptors injected prior to choice tests not only suppressed total intakes of the sweet solutions, but also reduced the preference for the sucrose-paired flavor in sham-feeding rats. Using real-feeding rats and a different conditioning paradigm, Hsiao and Smith [13] had previously reported that raclopride treatment reduced sucrose-conditioned flavor preferences.

The present study evaluated whether selective D_1 (SCH23390) or D_2 (raclopride) receptor antagonists administered during sham-feeding training altered the acquisition of the preference for the sucrose-paired flavor. Drug effects on the expression of the CS+ reference were also measured by injecting the rats with SCH23390 or raclopride prior to the CS+ vs. CS− choice tests. Four groups of rats were employed, three of which received vehicle, SCH23390 (200 nmol/kg) or raclopride (200 nmol/kg), respectively, before each of the one-bottle training sessions. Since both drugs were expected to reduce intakes during training, a fourth yoked control group received vehicle injections during training, but its sucrose and saccharin intakes were limited to the mean of the two drug groups. The data from this group were used to determine if any drug effect on preference conditioning was secondary to reduced intakes of the training solutions.

2. Methods

2.1. Subjects

Male albino Sprague–Dawley rats (350–400 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages and maintained on a 12:12-h light/dark cycle with Purina rat chow and water available ad libitum. Each rat was pretreated with chlorpromazine (3 mg/kg, ip) and anesthetized with ketamine HCl (100 mg/kg, im). Following a midline incision (4–7 cm) exposing the stomach outside of the skin and muscle, a stainless steel gastric fistula surrounded by mesh (Bard Marlex) was inserted into the greater curvature of the stomach, and was held in place by a purse-string series of sutures. The fistula was externalized through overlying skin and muscle, and an external stainless steel screw closed the fistula to prevent leakage of stomach contents. Two weeks of surgical recovery followed to allow for drug clearance.

2.2. Test solutions

The training solutions consisted of 16% sucrose (Domino Sugar) and 0.2% sodium saccharin (Sigma, St. Louis, MO) flavored with 0.05% unsweetened grape or cherry Kool Aid (General Foods, White Plains, NY). Half of the rats in each group had the cherry flavor added to the sucrose solution and the grape flavor added to the saccharin solution; the flavors were reversed for the remaining rats in each group. In the two-bottle preference tests, the cherry and grape flavors were each presented in a mixed solution containing 8% sucrose + 0.1% saccharin. The taste of sucrose was considered the US because it is strongly preferred to the taste of saccharin, and stimulates more sham feeding than saccharin at the concentrations employed in the present study [6,26,30]. The flavor added to the sucrose solution is referred to as the CS+, and the flavor added to the saccharin solution is referred to as the CS−. For initial sham-feeding training, an 8% maltodextrin solution was used (BioServ, Frenchtown, NJ), which has a distinctive taste to rats.

2.3. Initial training

The rats were placed on a food restriction schedule that maintained their body weights at 85–90% of their ad libitum level. They were initially trained to drink 8% maltodextrin solution from calibrated sipper tubes (100 ml, 1-ml gradations) while food and water were restricted, and then while food was restricted with water available ad

libitum. Prior to each daily 30-min session, the rats' gastric fistulae were opened, and their stomachs emptied by repeatedly flushing warm water (10–20 ml). At the end of the session, their stomachs were again flushed with warm water to minimize nutrient absorption, and the fistulae were closed. This sham-feeding procedure was repeated daily until all rats approached the sipper tubes with short (<1 min) latency, typically within 5 days. Food rations were given after the daily sham-feeding sessions.

2.4. One-bottle training

The first of four groups of rats (control group, $n=11$) received a vehicle injection (1 ml normal saline/kg body weight, sc) 30 min prior to each of the one-bottle training trials, while the second (D_1 group, $n=11$) and third (D_2 group, $n=12$) groups of rats received the D_1 antagonist, SCH23390 (Research Biochemicals, 200 nmol/kg, sc) and the D_2 antagonist, raclopride (Research Biochemicals, 200 nmol/kg, sc), respectively. These equimolar doses were chosen from preliminary pilot data indicating that these doses were at the high end of the dose range at which meaningful drinking of sucrose and saccharin solutions would occur under sham-feeding conditions. A fourth group of rats (yoked group, $n=13$) received vehicle injections each day, but was given only the mean amount of sucrose or saccharin solutions that was consumed under sham-feeding conditions by the D_1 and D_2 groups. The rats were given 10 one-bottle, sham-feeding training sessions (30 min/day) with the CS− saccharin solution presented on odd-numbered days, and the CS+ sucrose solution presented on even-numbered days. On Days 7–10, the rats had access to two sipper tubes, one containing the CS− or CS+ solution and the other containing water. This acclimated the rats to the presence of two sipper tubes during the choice tests. Water intake was negligible in these training trials. The position of the CS and water sipper tubes varied across days using a left–right–right–left pattern.

2.5. Two-bottle testing

Following training, the rats were given 10 two-bottle sham-feeding test sessions (30 min/day) with the CS+ and CS− flavors presented in mixed 8% sucrose + 0.1% saccharin solutions. The positions of the two sipper tubes were counterbalanced as described above. The four groups were treated identically and all had unlimited access to the test solutions and received the same sequence of drug injections. On Days 1 and 2, the rats received vehicle injections (1 ml/kg, sc) 30 min prior to the test sessions. Over the next 8 days, they received the short-acting antagonists, SCH23390 and raclopride at doses of 200 and 800 nmol/kg on every other day in counterbalanced order 30 min prior to the test sessions. On the four non-drug (to allow drug clearance) days, they received vehicle injections 30 min prior to the test sessions.

2.6. Statistics

CS intakes were recorded to the nearest milliliter. Intakes during training were evaluated by a randomized block analysis of variance with control, D_1, and D_2 groups as a between-subject variable, the CS− and CS+ conditions as one repeated-measure variable, and the 5 days of exposure as the second repeated-measure variable. Tukey-corrected comparisons ($P<.05$) detected significant effects. The yoked group was excluded from this initial analysis because their training intakes were limited to that of the drug groups. The two-bottle test data were evaluated with separate randomized block analyses of variance performed on CS+ and CS− intakes for the four groups as a function of pooled vehicle and antagonist dose treatments, total intakes as a function of vehicle and antagonist dose treatments, and percent CS+ intakes as a function of vehicle and antagonist dose treatments. Percent CS+ intakes were defined as the percentage of CS+ intake/ total intake.

3. Results

3.1. Drug effects on training intakes

Analysis of the training intakes of the control, D_1, and D_2 groups revealed significant differences in sham intakes among groups ($F(2,92)=356.78$, $P<.0001$), across training days ($F(4,184)=580.91$, $P<.0001$), between the CS+ and CS− conditions ($F(1,46)=2605.33$, $P<.0001$), and for each of the interaction conditions (P's $<.0001$). Overall, the three groups of rats drank almost four times more of the CS+ solution (19.9 ml) than the CS− solution (5.4 ml) during training (Fig. 1). Whereas intake of the CS−

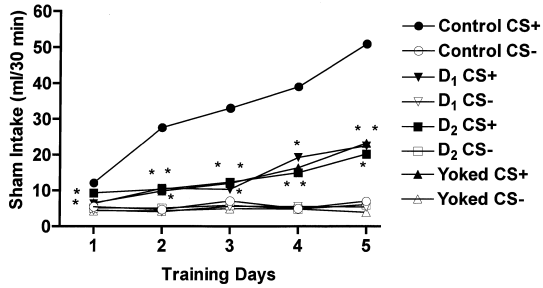

Fig. 1. Sham-feeding intakes (mean ± S.E.M.) in one-bottle training sessions (30 min) of a 16% sucrose solution containing one flavor (CS+, solid symbols) and a 0.2% saccharin solution containing a different flavor (CS−, open symbols); the flavors were 0.05% grape or cherry Kool Aid. The control group received systemic administration of saline (1 ml/kg, sc) 30 min prior to each training session (circles). The D_1 and D_2 groups received SCH23390 (200 nmol/kg, inverted triangles) and raclopride (200 nmol/kg, squares), respectively, 30 min prior to the training sessions. The yoked group (triangles) received saline injections, 30 min prior to training, but solution intakes were limited to the amounts consumed by the D_1 and D_2 groups. Significant differences between intakes of the D_1, and D_2 groups relative to the control group are indicated by asterisks (Tukey comparisons, $P<.05$).

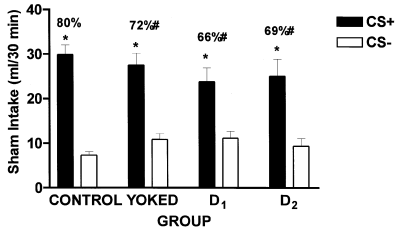

Fig. 2. Sham-feeding intakes (mean ± S.E.M.) of the CS+ and CS − flavored sucrose + saccharin solutions following vehicle treatment in the two-bottle tests in the four groups. Significant differences between CS+ and CS − intakes are denoted by asterisks (Tukey comparisons, $P < .05$). The percent CS+ intake (CS+ intake/total intake × 100) of each group is denoted; the # denotes significant differences relative to the control group.

solution in control rats remained stable over the 5 days of training, their intake of the CS+ solution significantly increased over training to a high of 50 ml (Fig. 1). Similarly, intake of the CS − solution in rats treated with either SCH23390 or raclopride remained stable over the 5 days of training, and failed to differ significantly from control rats. Although intakes of the CS+ solution increased over training days in rats receiving the D_1 and D_2 antagonists, they were significantly suppressed on all days relative to control rats. Finally, intakes of CS+ and CS − solutions in yoked rats closely matched that of the D_1 and D_2 groups, and therefore they consumed substantially less of the CS+ solution than did the control rats.

3.2. Drug effects on CS+ preference acquisition

In assessing whether the training treatment regimens altered the acquisition of the sucrose-conditioned flavor preference, the two-bottle CS+ and CS − intakes of the four groups during the vehicle treatment tests were compared. Analysis of these data revealed significant differences in the intake under vehicle treatment among the four groups ($F(3,138) = 5.79$, $P < .0009$), between CS+ and CS − intake ($F(1,46) = 1542.82$, $P < .0001$) and for the interaction between groups and intake conditions ($F(3,138) = 23.42$, $P < .0001$). Despite this interaction, CS+ intakes were significantly higher than CS − intakes in all four groups (Fig. 2), indicating the presence of strong sucrose-conditioned flavor preferences during vehicle treatment. The groups also failed to differ from each other in either the magnitude of CS+ intake or in the magnitude of CS − intake. However, there were group differences in the percent CS+ intakes: the percent CS+ intake of the control group (80%) was higher than that of the D_1 (66%), D_2(69%), and yoked (72%) groups. In contrast, the latter three groups did not significantly differ on this measure.

3.3. Drug effects on total test intakes of sucrose + saccharin solutions

Drug effects on the total intakes of the flavored sucrose + saccharin solutions during two-bottle tests were analyzed. Significant differences were observed in total intake during SCH23390 testing among the four groups ($F(3,184) = 18.51$, $P < .0001$), among doses ($F(2,368) = 2523.68$, $P < .0001$) and for the interaction between groups and doses ($F(6,368) = 19.57$, $P < .0001$). SCH23390 significantly and dose dependently reduced total intake in all groups such that intake following the 200-nmol/kg dose was significantly less than vehicle treatment, and intake following the 800-nmol/kg dose was significantly less than either the 0- or 200-nmol/kg dose (Fig. 3). Tukey comparisons revealed that the reductions in total intake following the 200-nmol/kg dose of SCH23390 was significantly greater in the D_2 and yoked groups relative to the control group.

Significant differences were observed in total intakes during raclopride testing among the four groups ($F(3,184) = 11.08$, $P < .0001$), among doses ($F(2,368) = 2275.15$, $P < .0001$) and for the interaction between groups and doses ($F(6,368) = 22.25$, $P < .0001$). Raclopride significantly and dose dependently reduced total intake in all groups such that the 200-nmol/kg dose reduced total intake relative to vehicle treatment, and the 800-nmol/kg dose reduced intake relative to the 0- and 200-nmol/kg doses (Fig. 3). The intake reduction produced by the 200-nmol/kg dose of raclopride was significantly greater in the yoked group relative to the control group.

3.4. Drug effects on CS+ preference expression

Analysis of the effects of SCH23390 on CS+ and CS − intakes during the two-bottle tests revealed that overall, the rats consumed more CS+ (14.1 ml) than CS − (7.1 ml) solutions ($F(1,46) = 1242.39$, $P < .0001$), and that there were significant interactions between SCH23390 doses and CS solutions ($F(2,92) = 1132.47$, $P < .0001$) and between groups and CS solutions ($F(3,138) = 37.96$, $P < .0001$). In all groups, both SCH23390 doses significantly reduced intake of the CS+ solutions relative to vehicle treatment, but failed to reduce CS − intake (Fig. 4). CS+ intake exceeded CS − intake following the 200-nmol/kg dose of

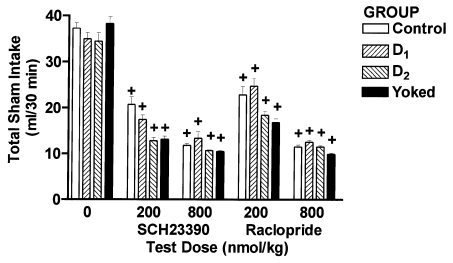

Fig. 3. Total sham-feeding intakes (mean ± S.E.M.) of both CS+ and CS − flavored sucrose + saccharin solutions during two-bottle tests following pretreatment with vehicle (mean of six tests), the D_1 antagonist, SCH23390 or the D_2 antagonist, at test doses of 200 and 800 nmol/kg. Rats in all groups had unlimited access to the solutions and received the same injection regimens. Differences (Tukey comparisons, $P < .05$) between intakes following vehicle and drug treatments are indicated by crosses.

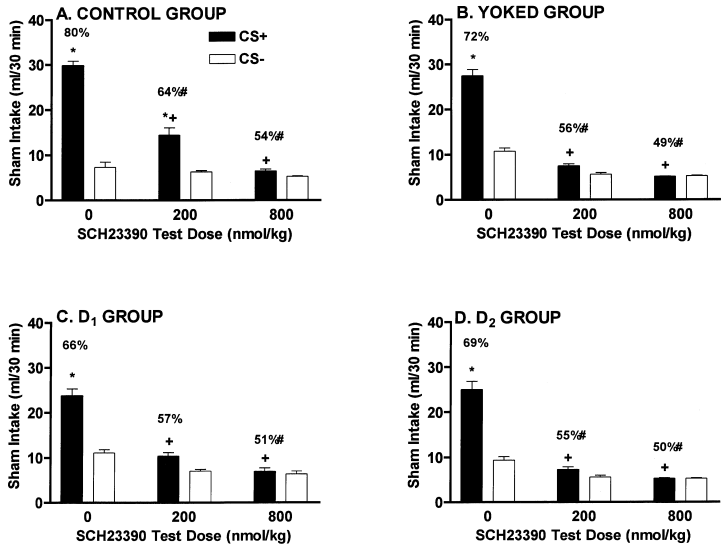

Fig. 4. Sham-feeding intakes of the CS+ and CS − flavored sucrose + saccharin solutions during two-bottle tests following treatment with vehicle and the D_1 antagonist, SCH23390 in each of the four groups. Significant differences between corresponding CS+ intakes following vehicle and drug treatments are indicated by crosses, whereas significant differences between CS+ and CS − intakes at each dose level are indicated by an asterisk (Tukey comparisons, $P < .05$). The # indicates significant differences in the percent CS+ intakes between vehicle and a given drug treatment.

SCH23390 in the control group (Fig. 4A), but not in either the yoked, D_1, or D_2 groups (Fig. 4B,C,D). In contrast, CS+ and CS − intakes did not differ in all four groups following the 800-nmol/kg dose of SCH23390. In assessing preference effects using percent of CS+ intakes, significant differences were observed among groups ($F(3,184) = 43.49$, $P < .0001$), across SCH23390 test doses ($F(2,368) = 938.77$, $P < .0001$) and for the interaction between groups and doses ($F(6,368) = 16.29$, $P < .0001$). Both test doses of SCH23390 significantly reduced the percent of CS+ intake in all four groups to the same degree (Fig. 4).

Analysis of the effects of raclopride on CS+ and CS − intakes during the two-bottle tests revealed that overall, the rats consumed more CS+ (15.1 ml) than CS − (7.5 ml) solutions ($F(1,46) = 1060.70$, $P < .0001$), and that there were significant interactions between raclopride doses and CS solutions ($F(2,92) = 886.20$, $P < .0001$) and between groups and CS solutions ($F(3,138) = 27.07$, $P < .0001$). In all groups, both raclopride doses significantly reduced intake of the CS+ solutions relative to vehicle treatment, but failed to reduce CS − intake (Fig. 5). All four groups displayed significantly greater CS+ intake than CS − intake

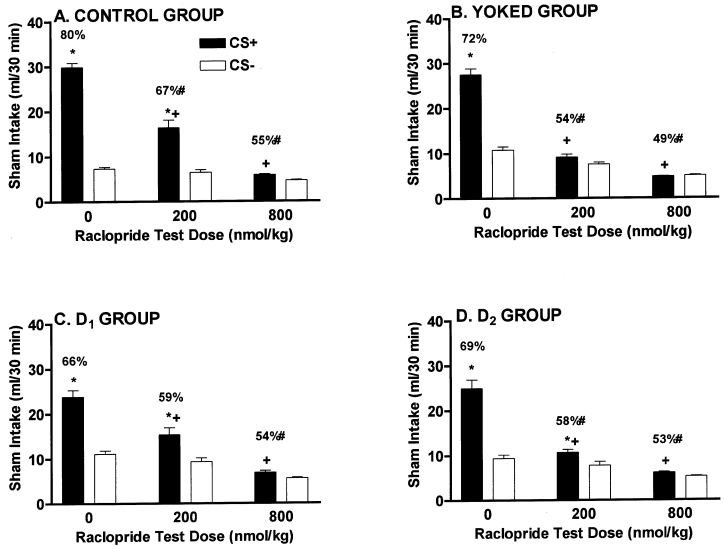

Fig. 5. Sham-feeding intakes of the CS+ and CS − flavored sucrose + saccharin solutions during two-bottle tests following treatment with vehicle and the D_2 antagonist, raclopride in each of the four groups.

following the 200-nmol/kg dose of raclopride, but displayed similar levels of CS+ and CS − intake following the 800-nmol/kg dose. In assessing preference effects using percent of CS+ intakes, significant differences were observed among groups ($F(3,184) = 36.47$, $P < .0001$), across raclopride doses ($F(2,368) = 641.75$, $P < .0001$) and for the interaction between groups and doses ($F(6,368) = 21.94$, $P < .0001$). The lower raclopride dose significantly reduced percent CS+ intake in the control, D_2, and yoked groups, but not in the D_1 training group. In contrast, the higher raclopride dose significantly reduced the percent CS+ intake in all four groups (Fig. 5).

4. Discussion

This experiment provided further confirmation that rats develop a reliable preference for a flavor paired with sucrose over a flavor paired with saccharin during one-bottle sham-feeding sessions as described previously [31,32]. Because the sham-feeding procedure minimized the post-ingestive actions of the sucrose solution, the CS+ preference is attributed to flavor–flavor conditioning. This experiment also confirmed that both the D_1 antagonist, SCH23390, and the D_2 antagonist, raclopride significantly reduced total intake of the combined sucrose–saccharin solutions during the two-bottle tests. Such effects are consistent with previous reports of decreased intake of palatable solutions under sham-feeding and real-feeding conditions following treatment with dopamine antagonists [9,17,20–23,28,32].

The present data also confirmed our prior findings [32] that SCH23390 and raclopride significantly and dose dependently reduced the intake and preference for the CS+ flavored solution without altering the intake of the CS − flavored solution in two-bottle tests conducted with food-restricted rats. The drug effects on the conditioned flavor preferences were evident using two statistical measures: comparisons of the absolute intakes of the CS+ and CS − solutions, and percent intakes of the CS+ solution. In evaluating the drugs' selective effects in reducing CS+ intakes during the two-bottle tests, it is important to note that CS − intakes were not so low as to preclude a suppression in intake: the CS − intakes of the four groups in the two-bottle vehicle tests ranged from 7 to 11 ml over the 30-min test. In our previous study [32], we obtained even higher CS − intakes (17 ml/30 min) in rats trained and tested under water-restricted conditions. As in the present study, raclopride treatment suppressed only CS+ intake during the two-bottle tests, and eliminated the CS+ preference at doses of 200–800 nmol/kg. In contrast, SCH23390 suppressed the intakes of both CS+ and CS − in the water-deprived rats. Nevertheless, the suppressive effect was much greater for the CS+ solution such that the CS+ preference was eliminated at doses of 200–800 nmol/kg. Thus, a "floor effect" on CS − intakes does not readily explain the selective reduction in CS+ intakes produced by raclopride and SCH23390. Rather, our present and prior [32] data are more consistent with the view that both dopamine receptor subtype antagonists interfere with the *expression* of conditioned flavor preferences in sham-feeding rats.

The new findings of the present study concern the effects of D_1 and D_2 receptor antagonists on the *acquisition* of sucrose-conditioned flavor preferences in sham-feeding rats. Rats treated with either SCH23390 or raclopride during one-bottle training displayed selective reductions in one-bottle intakes, with decreases noted on those days when they were exposed to the flavor paired with the 16% sucrose solution (CS+), but not on those days when they were exposed to the flavor paired with the 0.2% saccharin solution (CS −). This suggests that the equimolar (200 nmol/kg) dose of the antagonists decreased the reinforcing value of the sucrose, but not the saccharin solutions. It should be noted, however, that the CS+ intakes during one-bottle training were threefold higher than the CS − intakes for the D_1 and D_2 groups as compared to the sixfold difference in CS+ and CS − intakes displayed by the control group. Yet the D_1 and D_2 rats, despite their decreased training intakes of the CS+ solution, displayed significant preferences for the CS+ flavor over the CS − flavor in the subsequent drug-free two-bottle choice tests. The magnitudes of their CS+ preferences (66% and 69%, respectively) were significantly less than that of the control group (80%) rats, but importantly, did not differ from the yoked group (72%). This latter finding suggests that the reduced CS+ preferences observed in the drug groups, relative to the control group, may have been secondary to their reduced exposure to the CS+ solution during training. Strong evidence for a drug effect on preference acquisition would be provided if the drug groups showed a significantly reduced CS+ preference relative to the yoked group. Such a trend for a reduced CS+ preference was observed in the vehicle tests but was not significant. It is possible that flavor-preference acquisition would be prevented if the rats were trained with higher drug doses, but higher doses may reduce training intakes to a level that would preclude preference conditioning in the yoked control group. This issue requires further investigation with perhaps alternative methodologies (e.g., intraoral infusions of the CS solutions during training to maintain adequate intakes). With these limitations recognized, the present data does not provide clear evidence for a role of D_1 or D_2 receptors in the *acquisition* of a sucrose-conditioned flavor preference.

Exposure to either SCH23390 or raclopride during training also failed to alter the respective abilities of these antagonists to significantly reduce the *expression* of the conditioned flavor preferences. Although there were some minor differences in the degree of suppression observed in the various groups, overall, the D_1 group that received SCH23390 during training and the D_2 group that received raclopride during training, showed similar suppressions in CS+ preference as did the control and yoked groups

following SCH23300 or raclopride treatment in the two-bottle tests. There was a great deal of similarity in terms of both magnitude and potency between the two dopamine receptor antagonists in reducing the expression of sucrose-conditioned flavor preferences. There are multiple forms of both types of receptors [10], and there is considerable overlap in the localization of both receptor subtypes in the brain as revealed by autoradiographic techniques (e.g., Ref. [3]). Although one could potentially explain both effects by D_1–D_2 receptor interactions (e.g., Ref. [11]), this can only be determined using selective antagonists in discrete brain areas, and confirming these behavioral effects with biochemical measures.

In contrast to the present acquisition findings, Hsiao and Smith [13] reported that raclopride treatment during training reduced the preference for a flavor added to a sucrose solution in real-feeding rats. However, the design of their study differed importantly from the present experiment. Their rats were trained on alternate days with two differently flavored 10% sucrose solutions with intake of one solution preceded by a raclopride injection, and intake of the other solution preceded by a saline injection. In a subsequent drug-free choice test, the rats preferred the flavor that was previously paired with saline to the flavor that was previously paired with raclopride. This was taken as evidence that raclopride decreased the reinforcing potency of the sucrose solution. A potential problem with this design, however, is that a reduced flavor preference could be due to an aversive effect of the drug rather than to a specific attenuation of sucrose reinforcement. For this reason, the D_1 and D_2 groups in the present experiment were treated with the antagonist drugs on both CS+ and CS− training sessions so that any aversive drug effects would be associated with both CS flavors. Hsiao and Smith [13] argued against an aversion interpretation of their data. Assuming they are correct, their data can be taken as evidence that blocking D_2 receptors is sufficient to reduce the reinforcing potency of sucrose, whereas our data indicate that blocking D_2 receptors is not sufficient to eliminate the different reinforcing potencies of sucrose and saccharin.

In other words, it seems possible that raclopride and SCH23390 both reduced the reinforcing potency of sucrose throughout training, but since it could have had a similar effect on saccharin, the differential value of these two reinforcers was maintained, with the preference for CS+ left unchanged. A problem with this interpretation of the acquisition data, however, is that it does not lead us to expect that these drugs would have reduced selectively the expression of the preference for the CS+ flavor. Nevertheless, a dissociation between effects on acquisition and expression is not unprecedented. Elsewhere, we [7] have reported that naltrexone reduced the expression, but not acquisition, of a conditioned place preference reinforced by sucrose. The neural mechanisms underlying place preference conditioning, however, appear to differ from those involved in flavor–flavor learning since dopamine, but not opiate, antagonists modulate expression of flavor–flavor preferences.

The present acquisition data are consistent with Berridge and Robinson's [2] hypothesis that central dopamine systems do not mediate flavor learning in rats. They reported that rats with dopamine-depleting 6-hydroxydopamine lesions did not differ from controls in learning a flavor aversion conditioned by lithium chloride (LiCl) injections. However, Caulliez et al. [4] found that intrahypothalamic injections of a D_1 antagonist (SCH23390) but not a D_2 antagonist (sulpiride) disrupted the acquisition of a LiCl-conditioned taste aversion [4]. Given these conflicting findings, the role of dopamine in flavor aversion learning remains to be resolved. We are currently investigating the effects of D_1 and D_2 antagonists on the acquisition and expression of flavor preferences conditioned by intragastric sucrose infusions.

The present acquisition data with the D_1 and D_2 receptor antagonists extend our earlier observation that the general opioid antagonist naltrexone failed to block the acquisition of sucrose-conditioned preferences in sham-feeding rats [31]. In particular, although naltrexone suppressed CS+ intake during training, it did not reduce CS+ intake or preference in subsequent preference tests compared to the control group given unlimited access to the CS solutions during training. This differs from the present findings that the D_1 and D_2 antagonists reduced CS+ preference relative to the control group but not relative to the yoked group. Conceivably, naltrexone treatment enhances preference learning by allowing rats to acquire strong preferences despite reduced training intakes, but this requires confirmation. Naltrexone also failed to retard the expression of the sucrose-conditioned preference, which differs from the results obtained with the dopamine antagonists [32]. Since opioid and dopamine antagonists can have additive effects on suppressing the intake of sweet solutions, it may be that combined drug treatments are required to block the acquisition of sucrose-conditioned preferences (e.g., Ref. [25]). Alternatively, this type of flavor learning may be dependent upon other neurotransmitter systems.

In conclusion, the present experiment confirms prior reports that D_1 and D_2 receptor antagonists suppress the intake of sweet solutions, and more specifically blocks the expression of a flavor preference conditioned by the sweet taste of sucrose. Yet, the same antagonists did not display the same strong effects upon the acquisition of the sucrose-conditioned preference, relative to yoked controls, at the one training (200 nmol/kg) dose tested. Whether preference conditioning is blocked by high drug doses remains to be determined.

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