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Dopamine D_1 and D_2 antagonists reduce the acquisition and expression of flavor-preferences conditioned by fructose in rats

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Abstract

The effects of dopamine (DA) D_1 and D_2 receptor antagonists on the acquisition and expression of flavor-preferences conditioned by the sweet taste of fructose were examined. Food-restricted rats were trained over eight alternating one-bottle sessions to drink an 8% fructose solution containing one novel flavor (CS+) and a less preferred 0.2% saccharin solution containing a different flavor (CS −). Three groups of rats were treated daily with either vehicle (control group), SCH23390 (200 nmol/kg; D_1 group), or raclopride (200 nmol/kg; D_2 group) during training. Additional groups of vehicle-treated rats had their daily training intakes matched to that of the D_1 and D_2 groups. Preferences were assessed in two-bottle tests with the CS+ and CS − flavors presented in 0.2% saccharin solutions following doses of 0, 50, 200, 400, or 800 nmol/kg of either D_1 or D_2 antagonists. The D_1 and D_2 groups, unlike the control and yoked-control groups, failed to display a significant CS+ preference in the two-bottle tests following vehicle treatment. In addition, treatment with SCH23390 prior to the two-bottle tests blocked the expression of the CS+ preference in the control groups. Pretest raclopride treatment attenuated the CS+ preference at some dose levels. Raclopride also attenuated the preference for fructose in rats given two-bottle training with the CS+/fructose (CS+/F) and CS −/saccharin (CS −/S) solutions. These findings indicate that D_1 and D_2 antagonists block flavor-preference conditioning by sweet taste and that D_1, and to a lesser extent D_2, receptor antagonists attenuate the expression of a previously acquired preference.

Keywords: Flavor–flavor learning; Sweet taste; Saccharin, SCH23390; Raclopride

1. Introduction

Animals use flavor cues (taste, odor, and texture) to guide their selection of nutritious foods and avoidance of toxic foods (Capaldi, 1996). Flavor-preferences and aversions, in turn, are based in part on learned associations between the various flavor elements in the foods, flavor–flavor conditioning, and between the flavor cues and post-ingestive consequences, flavor–nutrient, and flavor–toxin conditioning. A primary example of flavor–flavor conditioning is the acquired preference for an arbitrary flavor cue (e.g., banana extract) added to a sweet solution (e.g., saccharin solution; Holman, 1975). The naturally preferred sweet taste is considered to be an unconditioned stimulus (US) that reinforces the animal's preference for the added flavor, which represents the conditioned stimulus (CS).

The potent reward value of sweet taste may result, in part, because sweet taste activates mesolimbic dopamine (DA) circuits that are implicated in the mediation of natural as well as drug rewards. It has long been known that DA antagonists suppress the intake of sweet solutions in rats (Geary and Smith, 1985; Muscat and Willner, 1989; Xenakis and Sclafani, 1981). Various findings suggest that this suppression results, in part, because DA antagonists reduce the reward value of sweet taste (Schneider, 1989; Smith, 1995) although other explanations have been proposed to account for drug effects on food intake and reinforcement (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Salamone et al., 1997). In addition to reducing the intake of sweet solutions, DA antagonists may also alter the ability of sweet solutions to reinforce the preference for other flavors. In particular, Hsiao and Smith (1995) reported

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that rats showed a reduced preference for a flavored 10% sucrose solution previously consumed while they were treated with the D_2 antagonist raclopride compared to a differently flavored sucrose solution previously consumed while they were treated with saline.

Sucrose can reinforce flavor-preferences based on its sweet taste as well as its postingestive nutritive actions through the processes of flavor–flavor and flavor–nutrient conditioning, respectively (Sclafani, 1995). Hsiao and Smith (1995) used brief (5 min) training sessions to minimize postingestive factors, but the amount of sucrose consumed in the training sessions may have had a postingestive reinforcing action. To separate the effects of drugs on flavor–flavor and flavor–nutrient learning, our laboratories have used sham-feeding and intragastric (IG) infusion procedures, respectively (Azzara et al., 2000, 2001; Yu et al., 1999, 2000a,b). With sham feeding, the ingested sucrose solution drains out of an open gastric fistula thereby minimizing postingestive nutrient actions (Weingarten and Watson, 1982). With the IG procedure, on the other hand, the sucrose is infused into the stomach thereby eliminating the sugar's taste as a conditioning factor (Sclafani, 1995). Yu et al. (2000a,b) used the sham-feeding procedure to determine the effects of DA antagonists on the acquisition and expression of flavor conditioning by the sweet taste of sucrose. Rats were treated with a D_1 antagonist (SCH23390), a D_2 antagonist (raclopride), or saline during sham-feeding training trials with different flavors added to a 16% sucrose solution or a less preferred 0.2% saccharin solution. In subsequent drug-free choice tests with both flavors presented in sucrose + saccharin solutions, the D_1 and D_2 groups displayed comparable preferences for the sucrose-paired flavor to those of saline-control rats that had their training intake limited to that of the drug groups, indicating a negligible effect on acquisition (Yu et al., 2000b). However, both antagonists dose-dependently reduced the preference for the CS+ flavor when administered prior to the choice test, indicating strong expression effects (Yu et al., 2000a).

The finding of Yu et al. (2000b) that DA antagonists did not block the acquisition of flavor conditioning by the sweet taste of sucrose would appear inconsistent with Hsiao and Smith's (1995) conditioning results as well as other studies suggesting that DA antagonists reduce the reward value of sweet taste (see Schneider, 1989; Smith, 1995). There are several procedural differences between the two conditioning studies that may account for the discrepant findings. In particular, Hsiao and Smith (1995) used a higher dose of raclopride and exposed their rats to less sucrose during training compared to the Yu et al. (2000b) study. In addition, the rats in the Hsiao and Smith (1995) study were given matched amounts of the raclopride-paired flavor and vehicle-paired flavor, whereas the drug-exposed rats in the Yu et al. (2000b) study were given unlimited access to the CS+ and CS − flavors, and both flavors were paired with raclopride.

In view of these considerations, the present study further investigated the effect of DA antagonism on flavor-preference learning produced by sweet taste. In this case, a conditioning procedure developed by Sclafani and Ackroff (1994) was used in which rats are trained to drink matched amounts of differently flavored 8% fructose and 0.2% saccharin solutions. This training procedure produces a robust preference for the fructose-paired flavor in a two-bottle choice test when both flavors are presented in 0.2% saccharin. Fructose, rather than sucrose or glucose, is used in this conditioning procedure because, unlike these other sugars, fructose has little or no postingestive reinforcing action during the short-term sessions. This is demonstrated by the failure of IG fructose infusions to condition a CS+ preference as well as by other findings (Sclafani and Ackroff, 1994; Sclafani et al., 1993, 1999). Thus, the preference for a flavor that is mixed into a fructose solution is considered to be a form of flavor–flavor learning based on the more preferred taste of 8% fructose relative to 0.2% saccharin (Sclafani and Ackroff, 1994).

2. Experiment 1

The first experiment determined if treating rats with a D_1 or D_2 antagonist (200 nmol/kg SCH23390 or raclopride; Yu et al., 2000b) during one-bottle training with flavored fructose and saccharin solutions attenuated their learning of a preference for the fructose-paired flavor. In addition to a vehicle-treated control group, which was trained like the two drug groups, two additional vehicle-treated groups had their training intakes matched to those of the D_1 and D_2 groups, respectively. Drug treatment was expected to reduce training intake of the flavored solutions, and the yoked-control groups allowed for a determination of the effect of reduced training intakes on flavor-preference learning. Following training, flavor-preferences were compared among the five groups with all rats treated with vehicle. The rats were then treated with various doses (50–800 nmol/kg) of SCH23390 or raclopride prior to the flavor-preference tests. In this way, drug effects on both the acquisition and expression of fructose-conditioned flavor-preferences could be assessed. An important feature of this experiment was that the rats in the drug groups were treated with SCH23390 or raclopride on both fructose and saccharin training days, so that any potential adverse drug effects would be associated with both flavors.

2.1. Methods

2.1.1. Subjects

All experimental protocols in the two experiments were approved by the Queens College Institutional Animal Care and Use Committee (Protocol #69) certifying that all subjects and procedures are in compliance with the National Institutes of Health Guide for Care and Use of

Laboratory Animals (Publication No. 85-23, revised 1985). Male albino Sprague–Dawley rats (260–300 g, Charles River Laboratories, Wilmington, MA, USA) 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. All testing took place in the rat's home cage during the midlight phase of the light–dark cycle in a normally illuminated animal colony room. Two weeks before testing began, the rats were placed on a food restriction schedule that maintained their body weights at 85–90% of their ad libitum level through the entire experiment.

2.1.2. Test solutions

The training solutions consisted of 8% fructose (Sigma, St. Louis, MO, USA) and 0.2% sodium saccharin (Sigma) flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY, USA). Half of the rats in each group had the cherry flavor added to the fructose solution and the grape flavor added to the saccharin solution; the flavors were reversed for the remaining rats. In the two-bottle preference tests, the cherry and grape flavors were each presented in a 0.2% saccharin solution. The fructose-paired flavor is referred to as the CS+ and the saccharin-paired flavor as the CS− because 8% fructose is preferred to 0.2% saccharin (Sclafani and Ackroff, 1994). CS+/F refers to the flavored fructose solution used in training, and CS+/S refers to the same flavor presented in saccharin during choice testing. The CS−/S refers to the flavored saccharin solution used in training and testing. For initial training, an 8% maltodextrin solution was used (BioServ, Frenchtown, NJ, USA).

2.1.3. Procedure

Rats were initially trained to drink an 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 except during the daily 2-h sessions. The sipper tube was mounted on the front of the cage held by a taut steel spring and was positioned so that the spout(s) entered the cage about 3–6 cm above the cage floor. This training procedure was repeated daily until all rats approached the sipper tubes with short (<1 min) latency, typically within 3 days. The limited food rations were given after each training session.

Three groups of rats were given 8 one-bottle training sessions (2 h/day) with 24 ml of the CS+/F solution presented on odd-numbered days, and 24 ml of the CS−/S solution presented on even-numbered days. On Days 5–8, the rats had access to two sipper tubes adjacently attached to the front of the cage, one containing the CS+/F or CS−/S solution, and the other containing water. This acclimated the rats to the presence of two sipper tubes used 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. Intakes were measured to the nearest 1 ml at 0.5 and 2 h during each session.

The rats in the first group (control group, $n=15$) received a vehicle injection (1 ml normal saline/kg body weight sc) 30 min prior to the one-bottle training trials, while rats in the second (D_1 group, $n=7$) and third (D_2 group, $n=7$) groups received the D_1 antagonist, SCH23390 (Research Biochemicals International, 200 nmol/kg sc), and the D_2 antagonist, raclopride (Research Biochemicals International, 200 nmol/kg, sc), respectively. These equimolar doses were chosen based upon their effects on sucrose–saccharin conditioned flavor-preferences in sham-feeding rats (Yu et al., 2000a,b). Two additional groups (D_1-yoked group, $n=8$; D_2-yoked group, $n=9$) received vehicle injections throughout the one-bottle training, but their intakes of CS+/F and CS−/S solutions were limited to the mean 2-h intakes of the D_1 (11 ml) and D_2 (19 ml) groups.

Following training, all groups were given 10 two-bottle choice test sessions (2 h/day) with the CS+/S and CS−/S solutions; intakes were unlimited in these tests. The positions of the two sipper tubes were counterbalanced as described above, and intake was measured after 0.5 and 2 h. The five groups received vehicle injections 30 min prior to the first two test sessions. Over the next 8 days, half of the control group ($n=7$), the D_1 group, and the D_1-yoked group received SCH23390 at ascending doses of 50, 200, 400, and 800 nmol/kg 30 min prior to the test sessions on odd-numbered days. The remainder of the control group ($n=8$), the D_2 group, and the D_2-yoked group received raclopride at ascending doses of 50, 200, 400, and 800 nmol/kg 30 min prior to the test sessions on the odd-numbered days. All groups were given vehicle injections on even-numbered test days.

2.1.4. Data analysis

Intakes during training were evaluated using analysis of variance for the control, D_1, and D_2 groups; the yoked-control groups were not included in this analysis because their intakes were matched to the drug groups. Preliminary analysis of the two-bottle data failed to reveal significant differences over the six vehicle sessions, and therefore the vehicle data were averaged over these sessions. A between-group analysis of the averaged vehicle data was performed to determine if the different conditions during training affected the expression of the CS+ vs. CS− preference. To determine if D_1 antagonism during two-bottle testing altered total intake or CS preference, analyses of variance was performed with the control, D_1, and D_1-yoked training groups across SCH23390 doses. Similar analyses were performed with the control, D_2, and D_2-yoked training groups across raclopride doses. Tukey corrected comparisons ($P<.05$) detected significant effects. The pattern of results for the 0.5-and the 2-h intake measurements were generally similar. To simplify presentation, only the 0.5-h data are presented in detail; the 2-h data are mentioned when they differ from the 0.5-h results.

2.2. Results

2.2.1. Drug effects on training intakes

Fig. 1A presents the one-bottle training intakes of the CS+/F and CS − /S averaged over the four sessions with each solution. Analysis of the 0.5-h data indicated that, overall, the groups differed in their CS intakes [$F(2,28) = 45.42$, $P < .0001$], that CS+/F intakes exceeded CS+/S intakes [$F(1,14) = 39.32$, $P < .0001$], and that there was an interaction between group and CS conditions [$F(2,28) = 16.52$, $P < .0001$]. Individual comparisons revealed that the control and D_2 groups did not differ in their CS intakes, and both groups consumed significantly more than the D_1 group did. Furthermore, both the control and D_2 groups, but not the D_1 group, consumed more CS+/F than CS − /S at the 0.5-h time point. There were no differences between the 2-h intakes of CS+/F and CS − /S across groups, and the D_1 group continued to drink less of the CS solutions than the control and D_2 groups [$F(2,28) = 23.94$, $P < .0001$]. Note that while the D_1-yoked rats consumed more than the D_1 rats during the initial 0.5 h of the session (Fig. 1A), the 2-h intakes of the two groups were well matched.

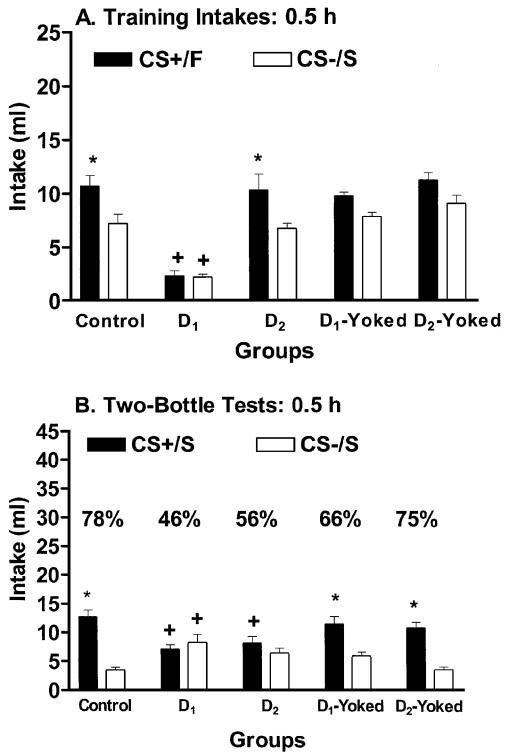

Fig. 1. (A) Intakes (mean ± S.E.M.) in one-bottle training sessions of an 8% fructose solution containing one flavor (CS+/F; four odd-numbered days) and a 0.2% saccharin solution containing a different flavor (CS − /S; four even-numbered days) after 0.5 h. The flavors were 0.05% grape or cherry Kool-Aid. The control group received systemic administration of saline (1 ml/kg ip) 30 min prior to each training session. The D_1 and D_2 groups received 200 nmol/kg doses of SCH23390 and raclopride, respectively, 30 min prior to the training sessions. The yoked-control groups received saline injections 30 min prior to training, but solution intakes were limited to the amounts consumed after 2 h by the D_1 (D_1-yoked: 11 ml) and D_2 (D_2-yoked: 19 ml) groups. Significant differences between CS+/F and CS − /S intakes within a group are denoted by asterisks (Tukey comparisons, $P < .05$). Significant differences in either CS+/F intake or CS − /S intake relative to the vehicle control training group are denoted by crosses (Tukey comparisons, $P < .05$). (B) Intake (mean ± S.E.M.) of CS+/S and CS − /S saccharin following vehicle treatment in the two-bottle tests in the five training groups after 0.5 h. The numbers atop the bars represent the percent of total intake consumed as CS+/S. Significant differences between CS+/S and CS − /S intakes within a group are denoted by asterisks (Tukey comparisons, $P < .05$). Significant differences in either CS+/S intake or CS − /S intake relative to the vehicle control training group are denoted by crosses (Tukey comparisons, $P < .05$).

2.2.2. Drug effects on CS+ preference acquisition

In assessing whether the different training regimens altered the acquisition of the fructose-conditioned flavor-preference, the two-bottle CS+/S and CS − /S intakes of the five groups were compared following vehicle treatment (Fig. 1B). Analysis of the 0.5-h data indicated that, overall, the rats consumed more CS+/S than CS − /S [$F(1,14) = 86.06$, $P < .0001$] and that there were significant Group × CS interactions [$F(4,56) = 25.56$, $P < .0001$], but no overall group effect on CS intakes. Individual comparisons revealed that the control, D_1-yoked, and D_2-yoked groups, but not the D_1 or D_2 groups, consumed significantly more CS+/S than CS − /S. Although the CS+/S preferences were somewhat weaker in the D_1-yoked and D_2-yoked groups (66% and 75%, respectively) compared to the control group (78%), these three groups did not differ in their CS+ or CS − intakes. The significant CS+/S preferences displayed by the yoked groups indicate that the lack of a CS+/S preference in the D_1 and D_2 groups was not due to their reduced CS intakes during training.

2.2.3. Drug effects on total test intakes

Analysis of total CS intakes during the two-bottle tests following SCH23390 treatment revealed significant differences across doses [$F(4,28) = 55.91$, $P < .0001$] and for the interaction between groups and doses [$F(8,56) = 2.14$, $P < .047$] but not among groups (Fig. 2A). SCH23390 significantly reduced total CS intakes in the control group following all doses. The D_1 and D_1-yoked groups displayed significant reductions in total CS intakes following all SCH23390 doses at 0.5 h.

Analysis of D_2 drug effects on the total CS intakes during the two-bottle tests revealed significant differences across doses [$F(4,32) = 23.73$, $P < .0001$], for the interaction between groups and doses [$F(8,64 = 9.43$, $P < .0001$], and among groups at the 0.5-h time point [$F(2,16) = 4.70$, $P < .025$; Fig. 2B]. Raclopride significantly reduced total CS intake in the control group at the 50, 400, and 800 nmol/kg doses. The D_2 group displayed significant reductions in total CS intakes following the 200 and 400 nmol/kg raclopride doses, whereas the D_2-yoked group displayed a significant intake reduction only following the 800 nmol/kg dose.

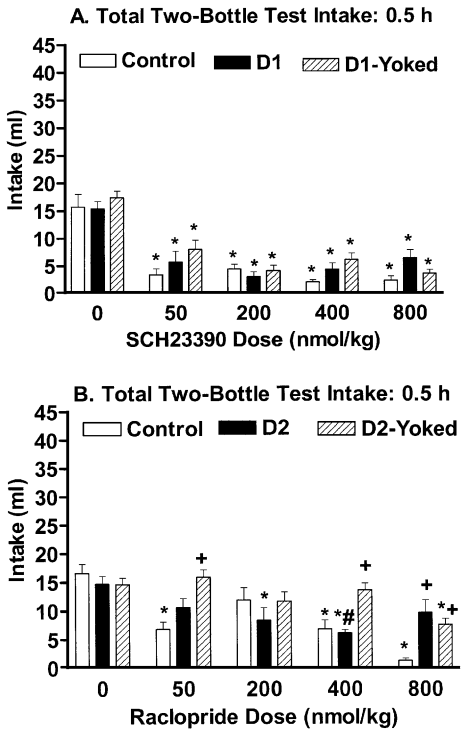

Fig. 2. Intake (mean ± S.E.M.) after 0.5 h of both CS+/S and CS − /S (total) solutions during two-bottle tests following pretreatment with vehicle or SCH23390 doses (50–800 nmol/kg) in the vehicle control, D_1, and D_1-yoked training groups (A) and following pretreatment with vehicle or raclopride doses (50–800 nmol/kg) in the control, D_2, and D_2-yoked training groups (B). Asterisks denote significant antagonist effects as compared to corresponding vehicle treatment (Tukey comparisons, $P<.05$). Crosses denote significant training group effects relative to the vehicle control training group (Tukey comparisons, $P<.05$). The pound sign denotes significant training group effects relative to the D_1-yoked group (Tukey comparisons, $P<.05$).

2.2.4. Drug effects on expression of CS+ preference

Since the control, D_1-yoked, and D_2-yoked groups showed a CS+/S preference, whereas the D_1 and D_2 groups failed to display CS+/S preferences, each of the groups were analyzed separately at each of the two time points.

Fig. 3 presents the two-bottle CS+/S vs. CS − /S intakes of the control, D_1, and D_1-yoked training groups following treatment with SCH23390. In control rats, significant differences were observed among SCH23390 doses [$F(4,30)=21.30$, $P<.0001$], between CS+/S and CS − /S solutions [$F(1,30)=5.33$, $P<.028$], and for the Dose × CS interaction [$F(4,30)=8.46$, $P<.001$]. The CS+/S preference (77%) of the control group expressed following vehicle was eliminated by all doses of SCH23390 (Fig. 3A). This was caused by significant drug-induced reductions in CS+/S but not CS − /S intake. In the D_1-yoked group, significant differences were observed among SCH23390 doses [$F(4,35)=21.90$, $P<.0001$], between CS+/S and CS − /S solutions [$F(1,35)=7.54$, $P<.0095$], and for the Dose × CS interaction [$F(4,35)=3.19$, $P<.025$]. The CS+/S preference (66%) of the D_1-yoked group following vehicle treatment was blocked by all SCH23390 doses (Fig. 3C). This was caused by significant drug-induced reductions in CS+/S but not CS − /S intake. In contrast, the D_1-trained rats did not display a CS+/S preference following vehicle treatment in two-bottle testing (46%), and significant differences were observed only among SCH23390 doses [$F(4,30)=11.61$, $P<.0001$]. The SCH23390 treatment did not alter their relative preference for the two CS flavors but significantly reduced CS+/S (200–400 nmol/kg) and CS − /S (50–400 nmol/kg) intakes (Fig. 3B). Thus, D_1 antagonism prior to two-bottle testing eliminated the expression of the fructose-conditioned CS+/S preference in those training groups (control and D_1-yoked) showing such a preference and reduced both CS+/S and CS − /S intakes in the training group (D_1) that failed to acquire a preference.

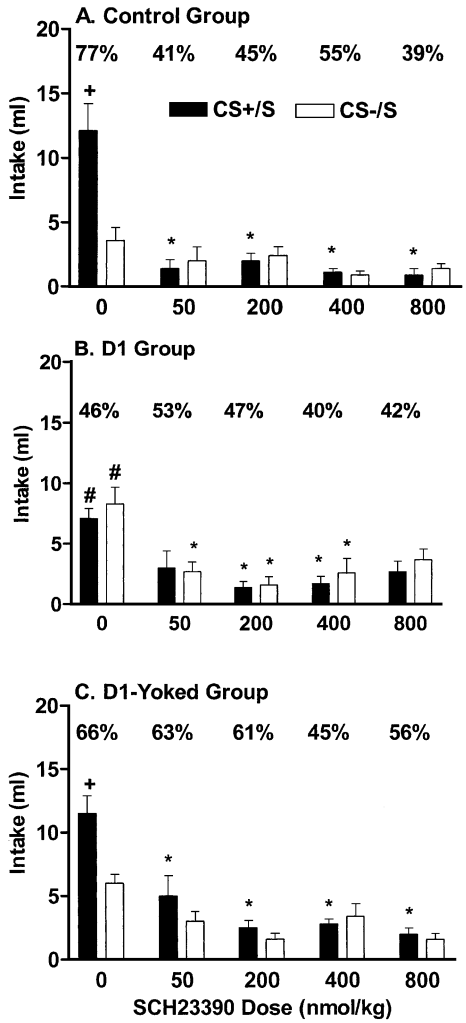

Fig. 3. Intake (0.5-h mean ± S.E.M.) of CS+/S and CS − /S solutions during two-bottle tests following treatment with vehicle and SCH23390 in the control (A), D_1 (B), and D_1-yoked (C) groups. Differences (Tukey comparisons, $P<.05$) between corresponding CS+ or CS − intakes following vehicle (0 nmol/kg) and drug treatments are indicated by asterisks, whereas the pound signs denote significant reductions in CS+ and CS − intake relative to the vehicle control-trained group in this and Fig. 4. The numbers atop the bars represent the percent of total intake consumed as CS+/S.

Fig. 4 presents the two-bottle CS+/S vs. CS − /S intakes of the control, D_2, and D_2-yoked training groups following treatment with raclopride. In control rats, significant differences were observed among raclopride doses [$F(4,35) = 14.62$, $P < .0001$], between CS+/S and CS − /S solutions [$F(1,35) = 41.63$, $P < .0001$], and for the Dose × CS interaction [$F(4,35) = 4.16$, $P < .007$]. As illustrated in Fig. 4A, the CS+/S preference of the control group persisted over most raclopride doses; only at the 200 nmol/kg dose did the control rats fail to drink more CS+/S than CS − /S. Raclopride significantly reduced CS+/S but not CS − /S intake following the 50, 400, and 800 nmol/kg doses. In the D_2-yoked group, significant differences were observed among raclopride doses [$F(4,40) = 5.41$, $P < .0014$], between CS+/S and CS − /S solutions [$F(1,40) = 34.62$, $P < .0001$], and for the Dose × CS interaction [$F(4,40) = 3.08$, $P < .027$]. The CS+/S preference (75%) of the D_2-yoked rats observed following vehicle treatment persisted following the 50 nmol/kg raclopride dose, but at the higher doses the rats no longer consumed more CS+/S than CS − /S (Fig. 4C). Raclopride significantly reduced CS+ intake only at the highest dose after 0.5 h, and its effects largely dissipated after 2 h such that CS+/S preferences were observed at the 50 and 400 nmol/kg doses. In contrast to the control and D_2-yoked groups, the D_2 group did not display a CS+/S preference following vehicle treatment in two-bottle testing (56%), and significant differences were observed only among raclopride doses at 0.5 [$F(4,30) = 4.50$, $P < .006$], and not after 2 h. Raclopride treatment significantly reduced CS+/S (200–400 nmol/kg) and CS − /S (200–400 nmol/kg) intakes at 0.5 h (Fig. 4B); these effects dissipated after 2 h. The D_2 rats showed no preference for the CS+/S at the 50, 200, and 800 nmol/kg doses, but unexpectedly they consumed more CS+/S than CS − /S at the 400-nmol/kg dose. The reason for this isolated preference is not clear. Thus, D_2 antagonism prior to two-bottle testing reduced intakes and had inconsistent effects on CS+/S preference in the two groups that acquired a preference for the fructose-paired flavor: the control group continued to prefer the CS+/S at all raclopride doses except 200 nmol/kg, whereas the D_2-yoked group displayed a CS+/S preference only at the 50 nmol/kg dose. The potency, duration, and magnitude of raclopride-induced effects upon these preferences thus appeared smaller than those induced by D_1 antagonism.

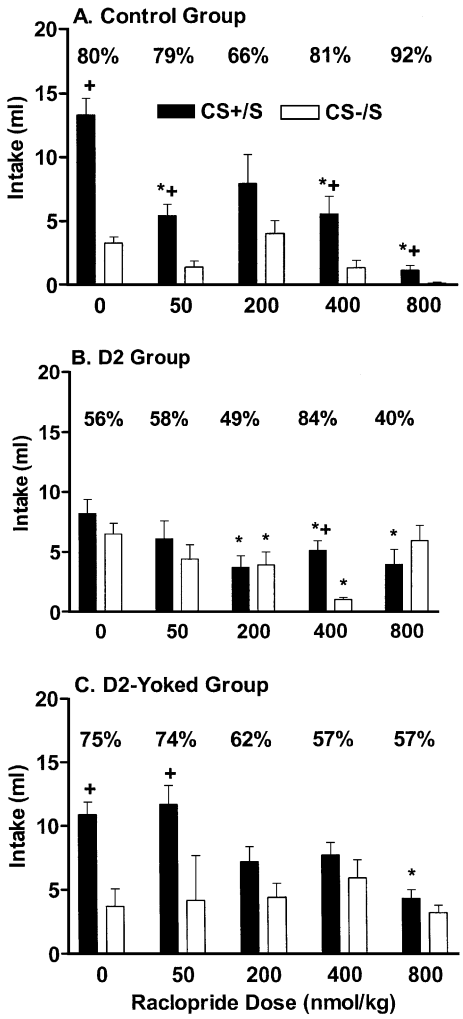

Fig. 4. Intake (0.5-h mean ± S.E.M.) of CS+/S and CS − /S solutions during two-bottle tests following treatment with vehicle and raclopride in the control (A), D_2 (B), and D_2-yoked (C) groups. Differences (Tukey comparisons, $P < .05$) between intakes of the CS+ and CS − solutions within a test are indicated by crosses. The numbers atop the bars represent the percent of total intake consumed as CS+/S.

3. Experiment 2

Experiment 1 revealed that both SCH23390 and raclopride treatment during one-bottle training blocked the acquisition of a fructose-conditioned flavor-preference. The raclopride effect was particularly impressive because, contrary to expectations, the drug did not reduce the training intakes of the flavored fructose and saccharin solutions. Furthermore, the D_2 rats, like vehicle-trained control rats, consumed significantly more fructose than saccharin during the initial 0.5 h of the training sessions, which suggests that raclopride treatment, while it blocked the ability of fructose to condition a flavor-preference, did not eliminate the unconditioned preference for fructose over saccharin. However, solution intakes in one-bottle tests do not always reflect preferences as measured in two-bottle tests (Sclafani, 1987), so that the training data of the first experiment do not provide a definitive assessment of raclopride's effect on fructose vs. saccharin preference. This issue was addressed in Experiment 2 in which control and D_2 groups received two-bottle access to flavored 8% fructose vs. 0.2% saccharin on half of the training sessions. Only the CS − was available during the remaining sessions to insure that the rats consumed sufficient amounts of CS − during training. A D_1 group was not included because the SCH23390-

treated rats in Experiment 1, unlike the raclopride-treated rats, did not consume more fructose than saccharin during the one-bottle training.

3.1. Methods

Male rats of similar strain, age, and source as those used in Experiment 1 were housed and pretrained with 8% maltodextrin as in the first experiment. One group (control group, $n=8$) received a vehicle injection (1 ml normal saline/kg body weight sc) 30 min prior to each of eight daily training trials, while a second group (D_2 group, $n=10$) received the D_2 antagonist, raclopride (200 nmol/kg). On odd-numbered training days, two-bottle training sessions (2 h/day) occurred with the rats receiving 24 ml each of the CS+/F and CS − /S. The left–right position of the CS solutions systematically varied over the days. On even-numbered days, the rats were given one-bottle training sessions (2 h/day) with 24 ml of the CS − /S available. Following training, the rats were given two-bottle test sessions (2 h/day) with the CS+/S and CS − /S solutions. The rats in both groups received vehicle injections 30 min prior to the two test sessions. As in the first experiment, intakes were recorded at 0.5 and 2 h during training and test sessions. Only the 0.5-h data are presented in detail although the 2-h data are described when they differ from the 0.5-h results.

3.2. Results

3.2.1. Drug effects on training intakes

Analysis of the two-bottle intakes on the odd-numbered training days revealed that, overall, the vehicle-treated control group consumed significantly more of the CS solutions than the D_2 group did after 0.5 h [$F(1,9)=12.53$, $P<.006$], that the rats consumed more CS+/F than CS − /S [$F(1,9)=46.01$, $P<.0001$], and that there was a significant interaction between groups and CS conditions [$F(1,9)=7.72$, $P<.021$] (Fig. 5A). Individual tests indicated that the control rats consumed more CS+/F than the D_2 group did in Session 7. The groups did not differ in their CS − /S intakes. The CS intakes increased over training sessions [$F(3,27)=21.38$, $P<.0001$], which was due primarily to an increase in CS+/F intake. Within-group comparisons revealed that the control rats consumed more CS+/F than CS − /S during all two-bottle sessions except the first one. The D_2 rats consumed more CS+/F than CS − /S during the last three 2-h sessions, although at the 0.5-h time point, the difference was significant only during the final training session. Overall, the preference for CS+/F over CS − /S for control rats (73–81%) was greater than that of the D_2 rats (53–75%; see Fig. 5A).

Analysis of the one-bottle CS-training intakes indicated that, overall, the control rats consumed more CS − /S than the D_2 rats [$F(1,9)=25.16$, $P<.0007$], that intakes in-

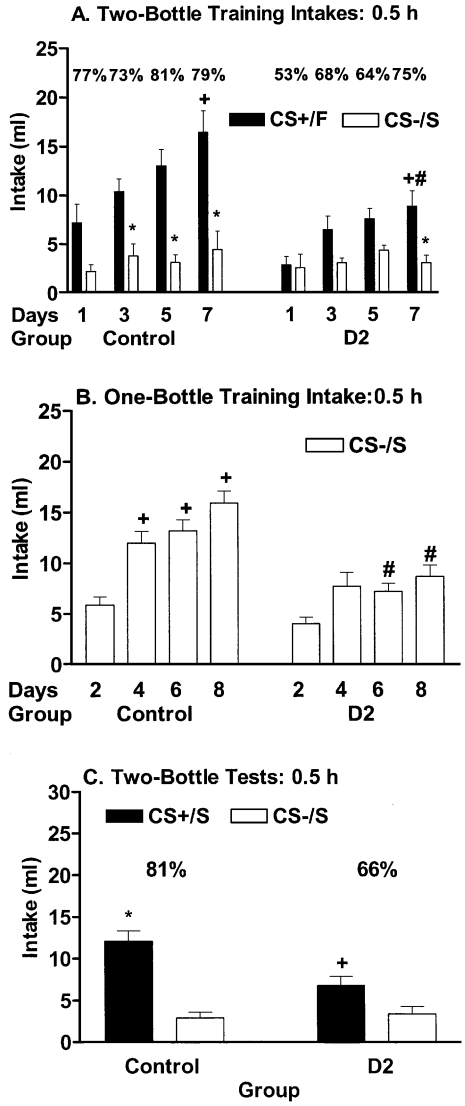

Fig. 5. Intakes (mean ± S.E.M.) in two-bottle training sessions of flavored 8% fructose (CS+/F) and 0.2% saccharin (CS − /S) solutions after 0.5 h during four odd-numbered days (A) and of CS − /S in one-bottle training sessions after 0.5 h on four even-numbered days (B). The control group received saline (1 ml/kg sc) 30 min prior to each training session, whereas the raclopride group received a 200 nmol/kg dose 30 min prior to the training sessions. Significant differences between CS+/F and CS − /S intakes within a group are denoted by asterisks (Tukey comparisons, $P<.05$). Significant differences in either CS+/F intake or CS − /S intake relative to the vehicle control training group are denoted by crosses (Tukey comparisons, $P<.05$). Significant differences in either CS+/F intake or CS − /S intake relative to the first day of training are denoted by pound signs (Tukey comparisons, $P<.05$). The numbers atop the bars represent the percent of total intake consumed as CS+/F. (C) Intake (mean ± S.E.M.) of CS+/S and CS − /S solutions during two-bottle testing sessions in control and D_2 groups after 0.5 h. Significant differences between CS+/S and CS − /S intakes within a group are denoted by asterisks (Tukey comparisons, $P<.05$). Significant differences in either CS+/S intake or CS − /S intake relative to the vehicle control training group are denoted by crosses (Tukey comparisons, $P<.05$). The numbers atop the bars represent the percent of total intake consumed as CS+/S.

creased over training sessions [$F(3,27) = 32.00$, $P < .0001$], and that there was Group × Session interaction [$F(3,27) = 3.28$, $P < .036$]. In particular, control rats consumed significantly more CS − /S than the D_2 rats did during the last three sessions (Fig. 5B). Over the 8 days of training, the rats consumed more CS − /S (intake totaled over one- and two-bottle training sessions at 2 h) than CS+/F, although this difference was significant only for the D_2 group [D_2: 100.9 ± 8.6 vs. 62.5 ± 5.9 ml; control: 106.5 ± 7.1 vs. 82.8 ± 3.8 ml].

3.2.2. Drug effects on CS+ preference acquisition

Fig. 5C presents the results of the CS+/S vs. CS − /S two-bottle test that followed vehicle treatment. Analysis revealed significant differences between groups [$F(1,9) = 9.50$, $P < .013$], between CS+/S and CS − /S intakes [$F(1,9) = 42.00$, $P < .0001$] and for their interaction [$F(1,9) = 9.20$, $P < .014$]. Individual tests indicated that the control rats consumed significantly more CS+/S than CS − /S; their percentage of CS+/S intakes was 81% (Fig. 5C). In contrast, the D_2 group failed to consume significantly more CS+/S than CS − /S. Compared to the controls, the D_2 rats drank less CS+/S; the groups did not differ in their intake of CS − /S.

4. General discussion

In confirmation of prior work (Sclafani and Ackroff, 1994), the control rats trained with flavored 8% fructose and 0.2% saccharin solutions displayed a significant (∼80%) preference for the fructose-paired flavor in choice tests with both flavors presented in saccharin solutions. This preference is attributed to the rats associating the CS+ flavor with the sweet taste of fructose rather than the sugar's postingestive actions. This assumption is based on findings showing that fructose has a relatively weak postingestive reinforcing effect (Sclafani and Ackroff, 1994; Sclafani et al., 1993, 1999). Also consistent with many prior reports (see Schneider, 1989; Smith, 1995), the control and drug groups reduced their intake of the fructose solution when injected with the DA receptor antagonists SCH23390 and raclopride prior to test sessions.

The new findings of the present study are that treating rats with SCH23390 or raclopride during one-bottle training blocked the development of the fructose-conditioned CS+ preference. In the case of the D_1 group, SCH23390 treatment significantly reduced the intakes of the flavored fructose and saccharin solutions during training. This reduction did not account for the failure of the D_1 group to acquire a CS+ preference, however, because the D_1-yoked rats, which were limited to the training intakes of the D_1 rats, displayed a significant CS+ preference. In Experiment 1, the D_2 and control groups consumed similar amounts of CS solutions during training, and therefore reduced CS intakes were not a factor in the D_2 group's failure to display a CS+ preference. In Experiment 2, the CS training intakes of the D_2 rats were somewhat less than that of the controls at the 0.5-h time point, but the 2-h intakes of the two groups did not differ. Taken together, these results indicate that the D_1 and D_2 antagonists did not prevent the acquisition of CS+ flavor conditioning because they reduced the exposure to the flavored fructose or saccharin solutions. Instead, the data suggest that the acquisition of flavor conditioning was inhibited because the drugs attenuated the reward value of the fructose taste.

Experiment 2 revealed that vehicle-treated control rats significantly preferred the CS+/F solution to the CS − /S solution during the two-bottle training sessions, which confirms prior findings that rats prefer 8% fructose to 0.2% saccharin in short-term "taste" tests (Sclafani and Ackroff, 1994). The D_2 rats injected with raclopride (200 nmol/kg) throughout training also preferred the CS+/F to the CS − /S during the training sessions, although their preference was attenuated relative to that of the control rats. This supports the idea that D_2 antagonism reduced the rats' attraction to the sweet taste of sugar. Nevertheless, the fact that the D_2 rats consumed more fructose than saccharin during training, but did not reliably prefer the fructose-paired flavor in testing, indicates that the acquisition of a flavor-preference conditioned by sweet taste is more susceptible to the D_2 drug antagonism than the unconditioned preference for the sweet taste itself.

The present results contrast with the findings of Yu et al. (2000b) that SCH23390 and raclopride did not prevent rats from acquiring a preference for a CS+ flavor paired with a sucrose solution over a saccharin-paired flavor. These discrepant results may be accounted for by the different conditioning procedures used in the two studies. In particular, while both studies paired the CS − flavor with a 0.2% saccharin solution, the CS+ flavor was paired with 16% sucrose (0.47 M) in our earlier work and with 8% fructose (0.44 M) in this study. Taste tests indicate the rats prefer sucrose to fructose over a range of isomolar concentrations (Sclafani and Mann, 1987). Furthermore, the rats in our earlier study sham fed the sucrose solution whereas the present rats real fed the fructose solution. This may have further increased the difference in the reward value of the two sugar solutions because some data suggest that postingestive satiety, experienced by a real-feeding but not a sham-feeding animal, attenuates the rat's attraction to carbohydrate solutions (Sclafani et al., 1994; Warwick and Weingarten, 1996). Another consequence of the sham-feeding procedure is that it allowed the rats to consume substantially more of the flavored sucrose solution than of the flavored saccharin solution during training, whereas the real-feeding rats of the present study consumed equivalent amounts of the fructose and saccharin training solutions. Thus, while treatment with the DA antagonists during training may have reduced the reward value of the flavored

sugar solutions in both studies, the drug effect may have been more pronounced with the real-fed fructose solution used in the present experiments than with the sham-fed sucrose solution used by Yu et al. (2000b). This interpretation predicts that DA antagonists would prevent flavor conditioning using a sucrose sham-feeding training procedure if a less concentrated, and therefore less preferred, sucrose solution was used.

In addition to preventing the development of an acquisition of a CS+ preference in the D_1 group, SCH23390 treatment blocked the expression of the CS+ preference in the control and D_1-yoked groups. This resulted from a reduction in CS+/S but not CS − /S intake during the two-bottle test sessions. Raclopride also selectively reduced CS+/S intake in the control and D_2-yoked groups, but this reduction was not as pronounced as that produced by SCH23390. Consequently, the control and D_2-yoked groups continued to prefer the CS+ flavor at some dose levels. This pattern of results, in general, agrees with previous data showing the D_1 and D_2 antagonism attenuated the expression of a flavor-preference conditioned by sucrose in sham-feeding rats (Yu et al., 2000a,b). The studies differed in that SCH23390 and raclopride had greater and weaker effects, respectively, on two-bottle test intakes and preferences in the current study than in our prior studies involving sucrose. Also, whereas the two drugs produced similar reductions in the expression of CS+ preference in the Yu et al. (2000a,b) studies, SCH23390 suppressed CS+ preference to a greater degree than raclopride in the present experiment. These different drug effects on two-bottle preference may be related to the fact that Yu et al. (2000a,b) tested flavor-preferences using a sucrose + saccharin solution that is more palatable than the plain saccharin solution used in the present study.

The present results extend the findings of Hsiao and Smith (1995) that DA receptor antagonism reduced the flavor-preference conditioning action of a sweet solution. In their study, rats preferred a flavored sucrose solution previously paired with vehicle injection over a different flavored sucrose solution previously paired with raclopride injection. A limitation of their conditioning procedure is that only one flavor is associated with the drug and thus adverse drug effects may contribute to the reduced flavor-preference. In the present study, however, both CS+ and CS − flavors were paired with the drug treatment making it unlikely that any adverse drug effects during training influenced the outcome of the flavor-preference test. An earlier study by Ettenberg and White (1981) observed that the D_2 antagonist pimozide blocked flavor-preference conditioning by lateral hypothalamic (LH) self-stimulation. In this experiment, rats that drank a coffee-flavored solution followed by the opportunity to bar press for LH self-stimulation subsequently preferred the flavored solution to plain water, whereas control rats not allowed to self-stimulate preferred water to the coffee solution. Other rats treated with pimozide during flavor/self-stimulation training failed to show a preference for the coffee solution. This finding may be relevant to the effects of D_2 antagonism on sugar-conditioned flavor-preferences obtained in the present study and by Hsiao and Smith (1995) because LH self-stimulation and food, sugars in particular, appear to act on the same (or highly similar) neural reward system(s) (Coons and White, 1977; Ono et al., 1985). Assuming that common systems are involved, the present findings predict that a D_1 antagonist would block flavor-preference conditioning by LH self-stimulation, and that both D_1 and D_2 antagonists would interfere with the expression of the flavor-preference based on LH self-stimulation.

As noted in the introduction, flavor-preferences can be reinforced not only by the sweet taste of sucrose, but also by its postingestive actions. Azzara et al. (2001) investigated the role of DA receptors in postingestive nutrient conditioning by pairing the intake of CS+ and CS − flavors presented in saccharin solutions with IG infusions of sucrose and water, respectively. Separate groups of rats were treated with SCH23390, raclopride, or vehicle during the conditioning sessions. Both DA antagonists reduced intake during one-bottle training, but only the D_1 antagonist blocked the development of a CS+ preference as revealed in two-bottle flavor tests conducted in the absence of the drugs. This contrasts with the effectiveness of both the D_1 and D_2 antagonists to block flavor conditioning by orally consumed fructose in the present experiment. Taken together, these findings indicate a differential involvement of DA receptor subtypes in the reinforcing actions of sweet taste and postingestive sugar reinforcement.

Other investigators have reported selective involvement of D_1 receptors in flavor aversion learning. In this case, water-restricted rats were trained to drink a sweet solution (saccharin or sucrose), which was followed by LiCl-induced toxicosis. The SCH23390 applied systemically or microinjected into either the lateral hypothalamus or nucleus accumbens retarded the development of a sweet-taste aversion (Caulliez et al., 1996; Fenu et al., 2001). In contrast, treatment with D_2 antagonists (raclopride or sulpiride) did not attenuate taste-aversion learning. These findings along with those of Azzara et al. (2001) suggest that D_1 receptors are involved in learning about both positive and negative postingestive consequences. However, some data suggest that different processes may be involved in preference and aversion learning. That is, in the Azzara et al. (2001) and present studies, flavor-preference conditioning was blocked by SCH23390 injected prior (15 or 30 min) to consumption of the CS solutions during training. In contrast, Fenu et al. (2001) reported that SCH23390 administered before (0 or 30 min) consumption of the CS solution was ineffective, and only SCH23390 injections given 5 min after CS intake (but before LiCl treatment) blocked taste-aversion learning. The preference and aversion conditioning procedures

differed in many respects, which may account for these discrepant results. Further work using common procedures is needed to elucidate D_1 receptor involvement in flavor-preference and aversion learning.

The present experiments add to a growing literature implicating DA receptors in flavor learning. These findings indicate that both D_1 and D_2 antagonists retard the acquisition of flavor-preference conditioning by the sweet taste of fructose. D_1 and, to a lesser extent, D_2 antagonists also attenuated the expression of a previously acquired flavor-preference. The results are consistent with the idea that DA antagonists reduce the rewarding properties of sweet taste (Schneider, 1989; Smith, 1995), although they do not exclude other interpretations of DA function. Also, as mentioned above, different DA subsystems may be involved in flavor–flavor and flavor–nutrient learning, and possibly in flavor-preference and flavor aversion learning. Berridge and Robinson (1998) have dichotomized food reward into hedonic (''liking'') and incentive (''wanting'') components. They proposed that DA is primarily involved in incentive aspects of food reward, whereas brain opioid systems mediate hedonic aspects of food reward. According to their model, DA is not critical for hedonic reward learning although this view is challenged by the findings that SCH23390 blocked flavor aversion learning as measured by intake and taste reactivity tests (Fenu et al., 2001). The present results also raise questions concerning the role of DA in hedonic reward learning since flavor–flavor conditioning is thought to involve hedonic processes (Breslin et al., 1990). This issue requires further investigation because the two-bottle choice tests used in the present study do not necessarily distinguish between hedonic and incentive components of learned flavor-preferences.

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