Published in final edited form as:

Physiol Behav. 2015 November 1; 151: 213–220. doi:10.1016/j.physbeh.2015.07.028.

Dopamine D1 and opioid receptor antagonists differentially reduce the acquisition and expression of fructose-conditioned flavor preferences in BALB/c and SWR mice

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Abstract

Sugar appetite is influenced by unlearned and learned preferences in rodents. The present study examined whether dopamine (DA) D1 (SCH23390: SCH) and opioid (naltrexone: NTX) receptor antagonists differentially altered the expression and acquisition of fructose-conditioned flavor preferences (CFP) in BALB/c and SWR mice. In expression experiments, food-restricted mice alternately (10 sessions, 1 h) consumed a flavored (e.g., cherry) 8% fructose + 0.2% saccharin solution (CS+) and a differently-flavored (e.g., grape) 0.2% saccharin solution (CS–). Two-bottle CS choice tests (1 h) occurred 0.5 h following vehicle, SCH (200 or 800 nmol/kg) or NTX (1 or 5 mg/kg). SCH, but not NTX significantly reduced CS+ preference in both strains. In acquisition experiments, 0.5 h prior to 10 acquisition training sessions, vehicle, SCH (50 nmol/kg), NTX (1 mg/kg) or Limited Control vehicle treatments were administered, followed by two-bottle CS choice tests without injections. SCH and NTX reduced training intakes in both strains. BALB/c mice displayed hastened extinction of the fructose-CFP following training with SCH, but not NTX. SCH eliminated fructose-CFP acquisition in SWR mice, whereas NTX hastened extinction of the CFP. These results are compared to previous drug findings obtained with sucrose-CFP in SWR and BALB/c mice, and are discussed in terms of differential effects of these sugars on oral and post-oral conditioning.

Keywords

SCH23390; Naltrexone; Orosensory (Flavor-Flavor) Learning; Genetic Variance

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1. Introduction

In addition to inborn preferences for sweet taste, learning plays an important role in modulating the appetite for sugar-rich foods. This is demonstrated in studies using the conditioned flavor preference (CFP) paradigm in which animals are trained to associate an arbitrary flavor (the conditioned stimulus, CS+) with a sugar solution and another flavor (the CS-) with water or a non-nutritive sweetener solution. Rats displayed a preference for a CS+ flavor mixed in a sucrose solution over a CS- flavor mixed in water (Mehiel & Bolles, 1988). Sucrose-CFP has been shown to be mediated by both orosensory and post-ingestive factors in rats with the orosensory component defined as flavor-taste conditioning produced by the sugar's sweet taste that is distinguished from flavor-nutrient conditioning produced by the sugar's post-ingestive effects (Sclafani, 1995). Flavor-nutrient learning has been extensively studied by training rats to associate a CS+ flavor with intragastric (IG) sucrose infusions and a CS- flavor with IG water infusions (Azzara et al., 2000, 2001). Flavor-taste learning has been studied by training rats to sham-feed a CS+ flavored sucrose solution and a CS- flavored saccharin solution; the sucrose, like the saccharin, drains out a gastric fistula and therefore has minimal post-ingestive nutritive actions (Yu et al., 1999, 2000a, 2000b). We have also investigated flavor-taste learning by training rats to "real-feed" a CS+ flavored fructose solution (Baker et al., 2003, 2004). The fructose-CFP was assumed to be reinforced by the sugar's sweet taste only because, unlike sucrose or glucose, IG fructose solutions failed to produce a CFP in rats using short daily training sessions (Sclafani et al., 1993, 1999; Ackroff et al., 2001).

Sugar intake in rats is mediated in part by brain dopamine (DA) (e.g., Geary and Smith, 1985; Muscat and Willner, 1989; Schneider et al., 1986; Sclafani et al., 1982) and opioid (e.g., Glass et al., 1996; Kirkham and Cooper, 1988; Levine et al., 1982, 1995; Rockwood and Reid, 1982; Sclafani et al., 1982) transmitter systems. In initial studies, we investigated the roles of DA and opioid systems in sugar-CFP. DA D1 or D2 receptor antagonism with SCH23390 (SCH) or raclopride, respectively, significantly attenuated the acquisition and expression of sucrose-CFP in sham-feeding rats (Yu et al., 2000a, 2000b), and the acquisition and expression of fructose-CFP in real-feeding rats (Baker et al., 2003), implicating these receptor subtypes in flavor-taste learning. DA D1, but not D2 receptor antagonism blocked CFP induced by IG sucrose in rats (Azzara et al., 2001), implicating this receptor subtype in flavor-nutrient learning. In contrast, opioid receptor antagonism with naltrexone (NTX) significantly reduced sugar intakes, but failed to affect the acquisition or expression of CFP in rats produced by sham-fed sucrose (Yu et al., 1999), real-fed fructose (Baker et al., 2004), or IG sucrose (Azzara et al., 2000).

In recent studies, we have extended our study of sugar intake and conditioning to inbred mouse strains. Pinhas et al. (2012) investigated strain differences in sucrose- and fructose-CFP. The food-restricted mice were trained (1 h/day) to real-drink CS+ flavored sugar and CS- flavored saccharin solutions in alternate one-bottle sessions and were then given two-bottle tests between the CS+ and CS- flavors both presented in saccharin solutions. SWR and BALB/c mice displayed the most robust and persistent CFP responses of the eight inbred strains tested. Other studies investigated the effects of DA and opioid receptor antagonists on sugar and saccharin intakes in these and other mouse strains. Whereas NTX

significantly reduced sucrose intake in BALB/c mice, it was rather ineffective in SWR mice (Table 1: Dym et al., 2007). In contrast, SCH produced comparable reductions in sucrose intake in BALB/c and SWR mice (Table 1). Raclopride inhibited sucrose intake in these and other murine strains only at very high doses, and was not examined in further studies (Dym et al., 2009). With respect to fructose, SCH and NTX were more effective in reducing intake in SWR than BALB/c mice (Table 1: Kraft et al., 2015). Both DA D1 and opioid receptor antagonists were very effective in reducing saccharin intake in both strains (Table 1: Kraft et al., 2015). Thus, selective strain differences were observed in the abilities of DA D1 and opioid antagonists to reduce nutritive and non-nutritive sweetener intakes in mice.

Dym et al., (2012) examined the effects of DA D1 and opioid receptor antagonism on sucrose-CFP in BALB/c and SWR mice using the conditioning paradigm developed by Pinhas et al. (2012). The expression of a previously-learned sucrose-CFP was significantly reduced in both BALB/c and SWR strains by SCH and NTX injections (Table 2A). In contrast, the acquisition of a sucrose-CFP was significantly reduced by NTX, but not SCH in BALB/c mice, and by SCH, but not NTX in SWR mice (Table 2D), indicating a double dissociation in the pharmacological effects on sucrose-CFP in the two strains.

In the present study, we investigated the effects of DA D1 and opioid receptor antagonists on the expression and acquisition of fructose-CFP in BALB/c and SWR mice. This was of interest because, as mentioned above, prior studies indicated that sucrose and fructose differ in their conditioning actions. Real-fed sucrose involves both flavor-taste and flavor-nutrient learning. In contrast, fructose-CFP was assumed to be reinforced only by sweet taste (flavor-taste learning) because IG fructose solutions failed to produce a CFP in rats as well as in C57BL/6 mice (Sclafani & Ackroff, 2012; Zukerman et al., 2013a,b). Thus, the disruptive effects of DA and opioid antagonists on fructose-CFP may differ from those observed with sucrose-CFP.

2. Materials and Methods

2.1. Subjects

Inbred BALB/c (Stock #000651) and SWR (Stock #000689) male mice (Jackson Laboratories, Bar Harbor, ME, 6 weeks of age, both strains ~25 g at arrival) were acclimated to the Queens College vivarium for one week in group (5 per cage) housing. The animals were then housed individually in plastic cages (30 × 20 × 15 cm) with stainless steel tops throughout the study, and maintained on a 12 h light/12 h dark cycle (lights off at 2000 h) at a constant temperature of 22°C. Chow (Lab Diet Mouse Chow 5015) and water were provided. Two weeks before testing began; the mice were placed on a food restriction schedule in which 2–3 g of chow was placed in their cages daily. The mice were weighed just prior to food-restriction, and their body weights were monitored until they gradually achieved 85–90% of their ad libitum level. The mice were maintained at that restricted level by limiting their intake throughout the experiment. The two strains maintained body weights between 28 and 35 g, and there were no weight differences between the strains. Individual mice were tested in one experimental paradigm. The experimental procedures were approved by the Queens College Institutional Animal Care and Use Committee certifying

that all subjects and procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Fructose-CFP Expression Procedure

Food-restricted mice were initially trained (1 h/day) to drink an unflavored 0.2% saccharin solution from a stainless steel sipper tube connected to a 10 ml plastic syringe (Dym et al., 2012; Pinhas et al., 2012). This training procedure was repeated daily until all mice sampled the sipper tubes with short (< 1 min) latency, typically within three days. Then two training solutions, 8% fructose + 0.2% saccharin and 0.2% saccharin, flavored with 0.05% unsweetened grape or cherry Kool-Aid (Kraft Foods, White Plains, NY) were presented. For half of the mice in each strain, the CS+ flavor added to the fructose-saccharin solution was cherry and the CS− flavor added to the saccharin solution was grape; the flavor-sweetener pairs were reversed for the remaining animals. In the two-bottle preference tests, the CS+ and CS− flavors were each presented in 0.2% saccharin solutions as in our prior studies (Dym et al., 2012; Pinhas et al., 2012). All testing took place in each mouse's home cage during the mid-light phase of the light:dark cycle. The limited food rations were given 1 h after each training and testing session. Note that the 8% fructose concentration combined with 0.2 % saccharin used in this study differs from that of our prior sucrose conditioning study (16%) (Dym et al., 2012) because we found that that 8% fructose and 0.2% saccharin conditioned a stronger preference than 16% fructose in SWR and BALB/c mice (Pinhas et al., 2012).

Twenty-four BALB/c and 23 SWR inbred mice received ten one-bottle training sessions (1 h/day) with 8 ml of the CS+/fructose solution presented on odd-numbered days, and 8 ml of the CS−/saccharin solution presented on even-numbered days. On days 9 and 10, all mice had access to a second sipper tube containing water. This familiarized them 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 the 2-bottle training and subsequent testing days using a left-right-right-left pattern. Solution intakes during training were measured by weighing (0.1 g) the sipper tubes before and after the 1-h sessions.

Following training, the mice were given six two-bottle choice test sessions (1 h/day) with access to the CS+ and CS− flavors mixed in 0.2% saccharin solutions. Thirty min prior to the first two days of two-bottle sessions, all mice were given an intraperitoneal (ip) vehicle (0.9% normal saline) injection. Then on subsequent days, 12 BALB/c and 10 SWR mice received 200 and 800 nmol/kg doses of SCH prior to two test sessions each as performed previously (Dym et al., 2012), with half tested with an ascending dose order and the remainder with a descending dose order. SCH was mixed at concentrations of 20 and 80 nmol/ml and administered at 10 ml/kg body weight. Additionally, 12 BALB/c and 11 SWR mice received 1 and 5 mg/kg doses of NTX as performed previously (Dym et al., 2012), with half tested with an ascending dose order and the remainder with a descending dose order. NTX was mixed at concentrations of 0.1 and 0.5 mg/ml and administered at 10 ml/kg body weight.

2.3. Fructose-CFP Acquisition Procedure

BALB/c and SWR mice received 10 one-bottle training sessions (1 h/day) with the CS+/Fructose solution presented on odd-numbered sessions, and the CS-/Saccharin solution presented on even-numbered sessions. Thirty min prior to each training session, intraperitoneal injections of vehicle (BALB/c: n= 6; SWR: n= 6), SCH (50 nmol/kg; BALB/c: n= 7; SWR: n= 7), or NTX (1 mg/kg; BALB/c: n= 6; SWR: n= 8) were administered as performed previously (Dym et al., 2012). To control for possible drug effects on CS training intake, which could influence conditioning, a fourth group (Limited Control: LTD CON) of BALB/c (n= 6) and SWR (n= 10) mice received vehicle injections throughout one-bottle training, but intakes of CS+/Fructose and CS-/Saccharin solutions were limited to the mean 1 h intakes of the SCH and NTX groups by exposing the animals during training to 15 min on the CS+ days and 20 min on the CS- days in each session. Following training, all mice were given six daily 2-bottle choice sessions (1 h/day) with access to CS+ and CS- flavored 0.2% saccharin solutions without injections. The positions of the CS+ and CS- solutions were counterbalanced across testing sessions, and the results were analyzed as mean 1 h intakes during successive pairs of sessions (referred to as Tests 1, 2 and 3).

2.4. Statistics

In expression, training intakes of the average of the five CS+/Fructose and five CS-/Saccharin sessions were determined for each strain, and evaluated for strain and CS differences using a two-way randomized-block analysis of variance (ANOVA). Expression of fructose-CFP in two-bottle preference tests was evaluated in a two-way ANOVA for strain differences following vehicle treatment. Vehicle intakes during the two-bottle preference tests were averaged over the two sessions and evaluated for strain differences in a two-way ANOVA. Subsequent two-ways ANOVAs evaluated drug effects within each strain (CS solution vs. Dose). Percent CS+ preference was calculated for each animal in each condition in the following manner: $\text{CS+ intake} / \text{Total Intake} \times 100$. Mean percent CS+ preference was calculated for each group by averaging the individual scores. Separate ANOVAs evaluated percent CS+ intakes as a function of drug dose for each strain as well as comparing strain differences. In acquisition, training intakes of the average of the five CS+/Fructose and five CS-/Saccharin sessions for the three (Veh, SCH, NTX) training groups were determined for each strain, and were evaluated for strain, drug group and CS differences using a three-way randomized-block ANOVA (Strain $\times$ Groups $\times$ CS). A three-way ANOVA compared the CS intakes of the four groups (Group $\times$ CS $\times$ Test). Separate two-way ANOVAs evaluated percent CS+ intakes of the four groups, whereas a 3-way ANOVA compared strain differences in percent CS+ intakes. When main or interaction effects were found, Tukey comparisons ($p < 0.05$) were employed to detect significant effects. Drug-induced reductions in the acquisition or expression of fructose-CFP are operationally defined as a significant reduction in percent CS+ intakes and/or a failure to observe significant differences between CS+ and CS- intakes in the two-bottle preference tests.

3. Results

3.1. DA D1 and opioid receptor antagonism and Fructose-CFP expression in BALB/c and SWR mice

During training, the SWR mice consumed more of the CS solutions than did the BALB/c mice ($F(1,23)=182.54$, $p<0.0001$). There was a CS $\times$ Strain interaction ($F(1,23)=86.48$, $p<0.0001$), however, and the BALB/c mice consumed more CS+ than CS- (1.02 ± 0.08 and 0.69 ± 0.08 ml/h, respectively) while the SWR mice consumed less CS+ than CS- (1.42 ± 0.08 and 2.24 ± 0.12 ml/h, respectively). In the vehicle two-bottle tests, both BALB/c and SWR strains consumed more CS+ than CS- ($F(1,23)=123.10$, $p<0.0001$). Note that the SWR mice consumed more CS+ than BALB/c (2.00 ± 0.21 vs. 0.88 ± 0.09 ml), while the two strains did not differ in CS- intakes (0.43 ± 0.06 vs. 0.18 ± 0.03 ml; CS $\times$ Strain interaction, ($F(1,23)=17.48$, $p<0.0001$). The two groups, however, displayed similar CS + percent preferences in the vehicle tests. Because the two strains showed dramatic differences in their CS+ two-bottle intakes, DA D1 and opioid antagonist effects on the expression of fructose-CFP were evaluated separately for each strain.

DA D1 Receptor Antagonism of Fructose-CFP Expression in BALB/c Mice—In the two-bottle tests following SCH, BALB/c mice overall consumed significantly more CS+ than CS- ($F(1,33)=53.29$, $p<0.0001$), and there were significant dose ($F(2,33)=35.12$, $p<0.0001$) and CS $\times$ Dose interaction ($F(2,33)=25.34$, $p<0.0001$) effects. CS+ intakes significantly exceeded CS- intakes following vehicle, but not the 200 or 800 nmol/kg SCH doses (Figure 1A). BALB/c mice consumed significantly less CS+ at both SCH doses compared to vehicle; CS- intakes failed to differ as a function of SCH dose (Figure 1A). Total CS intakes were significantly ($F(2,22)=29.54$, $p<0.0001$) lower following the 200 (0.28 ml) and 800 (0.25 ml) nmol/kg SCH doses relative to vehicle (1.1 ml). Percent CS+ intake was significantly reduced ($F(2,22)=5.83$, $p<0.01$) by the 200 (59%) and 800 (42%) nmol/kg SCH doses relative to vehicle (86%) (Figure 1A). These data indicate that the magnitude of fructose-CFP expression was reduced by DA D1 receptor antagonism in BALB/c mice.

DA D1 Receptor Antagonism on Fructose-CFP Expression in SWR Mice—In the two-bottle tests following SCH, SWR mice overall consumed significantly more CS+ than CS- ($F(1,27)=37.20$, $p<0.0001$) and there were significant dose ($F(2,27)=15.18$, $p<0.0001$) and CS $\times$ Dose interaction ($F(2,27)=10.47$, $p<0.001$) effects. CS+ intakes significantly exceeded CS- intakes following vehicle, but not the 200 or 800 nmol/kg SCH doses (Figure 1B). SWR mice consumed significantly less CS+ at both SCH doses compared to vehicle; CS- intake failed to differ as a function of SCH dose (Figure 1B). Total CS intakes significantly ($F(2,18)=15.64$, $p<0.0001$) declined following the 200 (1.14 ml) and 800 (0.74 ml) nmol/kg SCH doses relative to vehicle (2.76 ml). Percent CS+ intake was significantly reduced ($F(2,18)=4.01$, $p<0.05$) by the 200 (71%) and 800 (63%) nmol/kg SCH doses relative to vehicle (85%) (Figure 1B). These data indicate that the magnitude of fructose-CFP expression was reduced by DA D1 receptor antagonism in SWR mice.

Strain Differences in DA D1 Antagonism of Fructose-CFP Expression—

Analyses of strain differences in SCH-induced changes in percent CS+ intakes revealed significant differences among doses ($F(2,22)=12.52$, $p<0.001$), but not between strains or for the interaction between strains and doses, indicating that percent CS+ intakes following SCH treatment did not differ between strains.

Opioid Receptor Antagonism in Fructose-CFP Expression in BALB/c Mice—

In the two-bottle tests following NTX, BALB/c mice consumed significantly more CS+ than CS- ($F(1,33)=60.31$, $p<0.0001$), and there were significant dose ($F(2,33)=3.34$, $p<0.048$), but not CS $\times$ Dose interaction effects. CS+ intakes exceeded CS- intakes following vehicle and both NTX doses (Figure 2A). Total CS intakes significantly ($F(2,22)=4.55$, $p<0.05$) declined following the 5 mg/kg NTX dose (0.6 ml) relative to vehicle (1.0 ml) or the 1 (1.0 ml) mg/kg NTX dose. Percent CS+ intakes failed to differ following the 0 (79%), 1 (82%) and 5 (87%) mg/kg NTX doses (Figure 2A). These data indicate that the magnitude of fructose-CFP expression failed to be affected by opioid receptor antagonism in BALB/c mice.

Opioid Receptor Antagonism on Fructose-CFP Expression in SWR Mice—

In the two-bottle tests following NTX, SWR mice consumed significantly more CS+ than CS- ($F(1,30)=55.06$, $p<0.0001$), but neither dose nor CS $\times$ Dose interaction effects were significant. CS+ intakes significantly exceeded CS- intakes following vehicle and both NTX doses (Figure 2B). Total CS intakes failed to differ among vehicle (2.1 ml) and the 1 (2.1 ml) and 5 (1.5 ml) mg/kg NTX doses. Percent CS+ intakes did not differ following the 0 (77%), 1 (73%) and 5 (75%) mg/kg NTX doses (Figure 2B). These data indicate that the fructose-CFP expression failed to be affected by opioid receptor antagonism in SWR mice.

Strain Differences in Opioid Antagonism of Fructose-CFP Expression—There were no strain differences in the effects of opioid antagonism on percent CS+ intakes among doses, between strains or for the interaction between strains and doses.

3.2. DA D1 and Opioid receptor antagonism on Fructose-CFP acquisition in BALB/c and SWR mice

The two strains were initially evaluated for differences in one-bottle training intakes in the acquisition paradigm. Overall, the SWR mice (2.34 ± 0.08 ml/h) consumed significantly more of the CS solutions than did the BALB/c (1.54 ± 0.04 ml/h; $F(1,7)=73.64$, $p<0.0001$). Given this difference, the two-bottle preference test data in Vehicle, SCH, NTX and Limited Control groups were evaluated separately for each strain.

Fructose-CFP Acquisition in BALB/c mice—CS+/Fructose and CS-/Saccharin training intakes were significantly lower in the SCH and NTX groups relative to Vehicle, but not the Limited Control group (Figure 3A). In two-bottle tests, BALB/c mice overall consumed significantly more CS+ than CS- ($F(1,6)=63.32$, $p<0.0001$), but significant differences were observed among groups ($F(3,18)=3.47$, $p<0.05$), among tests ($F(2,12)=4.11$, $p<0.05$), and for the interactions between groups and tests ($F(6,36)=6.54$, $p<0.05$), groups and CS ($F(3,18)=4.50$, $p<0.05$) and tests and CS ($F(2,12)=4.11$, $p<0.05$).

CS+ intake was significantly higher than CS– intake across all tests in the Vehicle (Figure 4A) and NTX (Figure 4B) groups. However, in the SCH group (Figure 4C), CS+ intake was significantly higher than CS– intakes in Test 1, but not Tests 2 and 3, suggesting that DA D1 antagonism hastened the extinction of an acquired fructose-CFP in BALB/c mice. In the Limited Control group (Figure 4D), CS+ intake was significantly higher in Tests 1 and 2, but not Test 3, suggesting that the lower CS+ and CS– training intakes reduced the duration of the fructose-CFP preference in this strain. Percent CS+ preference significantly differed across tests ($F(2,12)=4.14$, $p<0.05$), but not among groups or for the interaction between groups and tests. Neither opioid nor DA D1 antagonism significantly altered percent CS+ preference across any test in BALB/c mice.

Fructose-CFP Acquisition in SWR mice—CS+/Fructose and CS–/Saccharin training intakes were significantly lower in the SCH and NTX groups relative to the Vehicle, but not the Limited Control group (Figure 3B). In two-bottle tests, SWR mice overall consumed significantly more CS+ than CS– ($F(1,9)=107.08$, $p<0.0001$), and significant differences were observed among tests ($F(2,18)=6.52$, $p<0.01$), and for the interactions between groups and tests ($F(6,54)=6.03$, $p<0.05$), between groups and CS ($F(3,27)=8.65$, $p<0.001$) and among groups, tests and CS ($F(6,54)=2.74$, $p<0.05$). CS+ intakes significantly exceeded CS– intakes across all tests in the Vehicle (Figure 5A) and Limited Control (Figure 5D) groups. In the SWR NTX group, CS+ intakes were significantly higher than CS– intakes in Tests 1 and 2, but not Test 3 (Figure 5B), suggesting hastening of extinction. In the SCH group, CS+ and CS– intakes did not differ from each other in all three tests (Figure 5C), suggesting elimination of the preference. CS+ intake (Tests 1, 2 and 3) in the SCH group and CS+ intake (Test 3) in the NTX group were significantly lower than corresponding vehicle CS+ intakes. Percent CS+ preference was significantly different among groups ($F(3,27)=11.23$, $p<0.0001$) and for the interaction between groups and tests ($F(6,54)=2.96$, $p<0.05$), but failed to differ across tests. Percent CS+ preferences in the SCH group in Tests 1 (40%) and 2 (46%) (Figure 5C) were significantly lower than corresponding Tests 1 (81%) and 2 (93%) of the Vehicle group (Figure 5A), and Tests 1 (74%) and 2 (72%) of the Limited Control group (Figure 5D). These data indicate that DA D1 receptor antagonism blocked the acquisition of fructose-CFP in SWR mice, and that opioid receptor antagonism hastened the extinction of the fructose-CFP in SWR mice.

Strain Differences in Fructose-CFP Acquisition—Analyses of percent CS+ intakes in the BALB/c and SWR groups in the acquisition of fructose-CFP revealed significant differences among training drug conditions ($F(3,27)=14.21$, $p<0.0001$) and for the interactions between strains and drug conditions ($F(3,27)=4.50$, $p<0.05$), between strains and tests ($F(2,18)=5.65$, $p<0.05$), between drug conditions and tests ($F(6,54)=4.14$, $p<0.01$), and among strains, drug conditions and tests ($F(6,54)=2.66$, $p<0.05$). SWR SCH mice displayed significantly lower percent CS+ intakes than BALB/c SCH mice (40% vs. 75%) in Test 1, and the difference approached significance for in Test 2 (46% vs. 61%). The SWR NTX mice displayed significantly lower percent CS+ intakes than BALB/c NTX mice (59% vs. 75%) in Test 3. In contrast, BALB/c Limited Control mice displayed significantly lower percent CS+ intakes than SWR Limited Control mice (57% vs. 78%) in Test 3,

indicating a hastening of extinction of the preference in the BALB/c strain under limited training conditions.

4. Discussion

The present study revealed strain differences in the pharmacological effects of DA and opioid antagonists on fructose-CFP in mice. The following sections will first describe the fructose conditioning effects observed in the BALB/c and SWR mice, and then discuss the drug effects on fructose-CFP in the two strains in comparisons with previous findings obtained with sucrose-CFP (Dym et al., 2012).

BALB/c and SWR Mice: Training and Preference Effects

Overall, the SWR mice consumed more CS training solutions than BALB/c mice. However, BALB/c mice consumed more CS+/Fructose than CS-/Saccharin while the SWR mice consumed more CS-/Saccharin than CS+/Fructose. These findings are consistent with the strain differences originally reported by Pinhas et al. (2012). As noted by these authors, the differential sugar and saccharin training intakes of the BALB/c and SWR mice may be related to genetic differences in sweet taste sensitivity. SWR mice have the sensitive form of the T1r3 sweet receptor and generally drink more sugar and non-nutritive sweeteners than do BALB/c mice that have the sub-sensitive form of the sweet receptor (Reed et al., 2004). The SWR mice may drink more CS-/Saccharin than CS+/Fructose because their saccharin intake is not limited by satiating actions of the sugar, whereas the BALB/c mice may drink less CS-/Saccharin during training because of their reduced sweetener preferences. Although SWR mice also consumed more CS+/Fructose in training than BALB/c mice, the two strains displayed similar CS+ preferences in the two-bottle tests: the BALB/c mice had CS+ preferences of 79–86%, and the SWR had CS+ preferences of 77–85%. These preferences are comparable to the CS+sucrose preferences displayed by BALB/c (74–85%) and SWR (75–78%) mice in our prior drug study (Dym et al., 2012: Table 2), and confirm our original mouse CFP findings (Pinhas et al., 2012). Thus, comparisons between sugars and mouse strains are appropriate because the CFPs observed in the vehicle non-drug expression tests were very similar.

DA D1 Receptor Antagonism and Fructose-CFP Expression

In BALB/c mice, SCH eliminated fructose-CFP expression (42–59%) relative to vehicle (86%), an effect that was stronger than the ability of SCH (68–73%) to reduce the expression of sucrose-CFP relative to vehicle (85%) in BALB/c mice (Dym et al., 2012; Table 2A and B). Thus, the magnitude of DA D1 antagonist effects appeared more pronounced on fructose-CFP (using a 8% fructose + 0.2% saccharin CS+ stimulus) relative to sucrose-CFP (using a 16% sucrose CS+ stimulus) in BALB/c mice. This may be due to a greater reliability on flavor-taste (e.g., in fructose) processes relative to flavor-nutrient (e.g., observed in sucrose) in BALB/c mice.

In SWR mice, SCH induced comparable reductions of fructose-CFP expression (63–71%) relative to vehicle (85%) and sucrose-CFP expression (61–66%: Dym et al., 2012) relative to vehicle (75%) (Table 2A and B). This contrasts with the BALB/c results and the notion

that SCH differentially affects flavor-taste and flavor-nutrient conditioning effects. However, recent findings indicate that both fructose and sucrose have post-ingestive conditioning actions in SWR mice (Huang et al., 2014). Previous studies in rats (Ackroff and Sclafani, 2001; Sclafani et al., 1993, 1999) and more recently in C57BL/6J mice (Sclafani & Ackroff, 2012; Zukerman et al., 2013a,b) indicated that fructose has little or no post-ingestive flavor conditioning actions. Subsequent experiments with FVB mice, however, revealed that this strain learns to prefer flavors based on post-ingestive fructose effects (Sclafani et al., 2014). For example, FVB mice, unlike C57BL/6J mice, learned to prefer a fructose-paired flavor over a different flavor paired with a “sweeter” non-nutritive sucralose-saccharin mixture. Using this paradigm, we observed that SWR mice, like FVB mice, display evidence of post-ingestive fructose conditioning (Huang et al., 2014). In contrast, BALB/c mice were like C57BL/6J mice in their failure to show post-ingestive fructose conditioning (Huang et al., 2014). These new findings suggest that SCH had similar effects on sucrose- and fructose-CFP in SWR mice because both sugars involve flavor-taste and flavor-nutrient learning processes. In contrast, the drug had dissimilar effects in BALB/c mice because in this strain, only sucrose involves both flavor-taste and flavor-nutrient learning processes.

Opioid Receptor Antagonism and Fructose-CFP Expression

In contrast to SCH, NTX failed to alter the expression of fructose-CFP in BALB/c or SWR mice (Table 2B). This contrasts with our prior finding that NTX promoted the extinction of a sucrose-CFP preference with repeated testing in BALB/c mice and even more so in SWR mice (Dym et al., 2012; Table 2A). These findings suggest that the opioid antagonist is more effective in suppressing the expression of sucrose-CFP than fructose-CFP. However, the present and prior study used different sugar concentrations during training (8% vs. 16%) which may have influenced drug effects on the persistence of the conditioned CS+ preferences. This issue requires further study.

DA D1 receptor antagonism of Fructose-CFP acquisition

Administration of the DA D1 receptor antagonist, SCH blocked the acquisition of a fructose-CFP in SWR mice in that they failed to significantly prefer the CS+ over the CS– in Tests 1–3 (40%–46%–64%; Table 2E). This pattern is similar to SCH-induced inhibition of sucrose-CFP acquisition in SWR mice although, as in the present experiment, the SWR-sucrose trained mice displayed an increase in CS+ preference in their last two-bottle test (61%–74%; Table 2A; Dym et al., 2012). In contrast, the SCH BALB/c mice displayed a significant 75% fructose-CFP preference in the first test (Table 2E) that was similar to the previously-observed (Dym et al., 2012) initial 77% sucrose CS+ preference in the first test (Table 2D). However, the SCH BALB/c mice subsequently lost the fructose-CFP preference in Tests 2 and 3 (61%–59%; Table 2E). This is in contrast to the sucrose-trained BALB/c mice which maintained their CS+ preference in their second (and last) test (80%; Table 2D; Dym et al., 2012). These data suggest that SCH administered to BALB/c mice during training hastens extinction of a fructose-CFP, but not of a sucrose-CFP. However, a potential caveat for this interpretation concerns the behavior of the Limited Control group of BALB/c mice included in this study. This group, which had their training intakes matched to the SCH and NTX groups, displayed significant CS+ preferences in the first (74%) and

second (75%), but not the third (57%) tests, suggesting that the persistence of fructose-CFP in BALB/c mice was weakened by limiting their CS intakes during training. The BALB/c and SWR strains were chosen for their respective robustness and persistence in displaying sucrose- and fructose-CFP, but these effects were observed in animals that did not have profound restrictions on training intake (Pinhas et al., 2012).

Opioid receptor antagonism of Fructose-CFP acquisition

NTX failed to alter the acquisition of fructose-CFP in BALB/c mice in that they displayed significant CS+ preferences in Tests 1–3 (88%–87%–75%; Table 2E). In contrast, NTX BALB/c mice trained with sucrose failed to show a CS+ preference in Test 1 (54%) although a weak preference emerged in the second test (66%; Table 2D; Dym et al., 2012). The different NTX effects on fructose- and sucrose-CFP in BALB/c mice cannot be explained by any differential inhibitory actions of NTX on sucrose and fructose intake, *per se* (Dym et al., 2007; Kraft et al., 2015). Conceivably, the stronger NTX inhibitory effect on sucrose-CFP than fructose-CFP may be related to sucrose, but not fructose having a post-ingestive conditioning action in this strain. Further studies should examine whether IG sucrose produces a CFP in BALB/c mice like those observed in 129P3 and C57BL/6J strains (Sclafani and Glendinning, 2005), and if so, whether IG sucrose-CFP acquisition in BALB/c mice is altered by opioid receptor antagonism.

The NTX SWR mice displayed significant CS+ preferences in Tests 1 and 2 but not in Test 3 (72%–76%–59%) unlike the persistent CS+ preferences displayed by the Vehicle and Limited Control groups (Table 2E, Figure 5). In contrast, NTX SWR mice trained with sucrose displayed strong and persistent CS+ preferences (83%–85%), like their Vehicle controls (86%–89%), although note that these mice were not given a third choice test. Thus, it appears that NTX has opposite effects on sugar conditioning in SWR and BALB/c mice trained with fructose and sucrose, although this conclusion is not definitive given procedural differences in the two sugar studies.

Acknowledgments

This research was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK071761 to AS and RJB and PSC/CUNY Grants 41-62438, 42-336 and 43-232 to RJB. YY was a student in the Macauley Honors College at Queens College. We thank V. Acosta, N. Borukhova, L. Borukhov, M. Elkomos Botros and L. Luu for their technical assistance.

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Highlights

- Fructose-conditioned flavor preferences (CFP) were observed in BALB/c and SWR mice.
- Dopamine (DA) D1 antagonism reduced fructose-CFP expression in both strains.
- Opioid antagonism failed to affect fructose-CFP expression in both strains.
- Extinction of BALB/c fructose-CFP acquisition was hastened by DA D1 antagonism.
- SWR fructose-CFP acquisition was blocked by DA D1 antagonism.

Figure 1. (Fructose-CFP Expression). Intakes (mean $\pm$ SEM, g/1 h) of CS+ and CS- solutions in two-bottle tests in BALB/c (upper panel) and SWR (lower panel) inbred mice receiving systemic injections of the DA D1-like antagonist, SCH23390 at doses of 0, 200 and 800 nmol/kg 30 min prior to testing. Significant differences are denoted between CS+ and CS- intake within an injection condition (*) and between CS+ intake following a drug dose relative to the vehicle treatment (+). The percentages of CS+ intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (+) noted.

Figure 2.

(Fructose-CFP Expression). Intakes (mean +SEM, g/1 h) of CS+ and CS- solutions in two-bottle tests in BALB/c (upper panel) and SWR (lower panel) inbred mice receiving systemic injections of the general opioid antagonist, naltrexone at doses of 0, 1 and 5 mg/kg 30 min prior to testing. Significant differences are denoted between CS+ and CS- intake within an injection condition (*) and between CS+ intake following a drug dose relative to the vehicle treatment (+). The percentages of CS+ intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (+) noted.

Figure 3.

(Fructose-CFP Acquisition Training). Intakes (mean $\pm$ SEM, g/1 h) of CS+/Fructose and CS-/Saccharin solutions in BALB/c (upper panel) and SWR (lower panel) mice pretreated 30 min earlier with vehicle (Veh), SCH23390 at a dose of 50 nmol/kg (SCH), naltrexone at a dose of 1 mg/kg (NTX) or vehicle and limited to drug-induced intake (LTD CON).

Significant differences in CS+/Fructose or CS-/Saccharin intakes following SCH or NTX relative to corresponding Veh (+) are denoted.

Figure 4.

(Fructose-CFP Acquisition). Intakes (mean +SEM, g/1 h) of CS+ and CS- solutions during two-bottle Tests 1, 2 and 3 in BALB/c mice receiving Veh (Panel A), naltrexone (Panel B), SCH23390 (Panel C) or the LTD CON condition (Panel D) during training. Significant differences are denoted between CS+ and CS- intake (*) and for drug treatment relative to corresponding Veh (+). The percentages of CS+ intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (+) noted.

Figure 5. (Fructose-CFP Acquisition). Intakes (mean +SEM, g/1 h) of CS+ and CS- solutions during two-bottle Tests 1, 2 and 3 in SWR mice receiving Veh (Panel A), naltrexone (Panel B), SCH23390 (Panel C) or the LTD CON condition (Panel D) during training. Significant differences are denoted between CS+ and CS- intake (*) and for drug treatment relative to corresponding Veh (+). The percentages of CS+ intake over total intake are denoted above each pair of values with significant differences relative to vehicle treatment (+) noted.

Table 1

Linear regression values (ID40) of SCH23390 (SCH, nmol/kg)- and naltrexone (NTX, mg/kg)-induced inhibition (60 min) of saccharin (0.2%), fructose (8%) and sucrose (10%) intakes in BALB/c and SWR mice.

Condition	BALB/c	SWR	BALB/c	SWR
	SCH	SCH	NTX	NTX
Saccharin ^a	<50 nmol/kg	<50 nmol/kg	0.9 mg/kg	0.02 mg/kg
Fructose ^a	1234 nmol/kg	298 nmol/kg	4.99 mg/kg	2.59 mg/kg
Sucrose ^b	638 nmol/kg	811.5 nmol/kg	3.61 mg/kg	6.86 mg/kg

^aData from Kraft et al., 2015

^bData from Dym et al., 2007 and 2009

TABLE 2

Summary of dopamine (DA) D1 (SCH23390: SCH) and opioid (naltrexone: NTX) receptor antagonist effects upon the expression and acquisition of conditioned flavor preferences (CFP) elicited by sucrose and fructose in BALB/c and SWR mice.

CFP Condition	SCH (nmol/kg)	SCH (nmol/kg)	NTX (mg/kg)	NTX (mg/kg)
	BALB/c	SWR	BALB/c	SWR
A. Sucrose-CFP	0: 85%	0: 75%	0: 74%	0: 78%
Expression Effects	200: 73%*	200: 66%*	1: 76%	1: 63%*
	800: 68%*	800: 61%*	5: 63%*	5: 63%*
B. Fructose- CFP	0: 86%	0: 85%	0: 79%	0: 77%
Expression Effects	200: 59%*	200: 71%*	1: 82%	1: 73%
	800: 42%*	800: 63%*	5: 87%	5: 75%
C. Sucrose-CFP	Veh: 85%–77%	Veh: 86%–89%	Veh: 85%–77%	Veh: 86%–89%
Acquisition Effects	SCH: 77%–80%	SCH: 61%* –74%	NTX: 54%* –66%	NTX: 83%–85%
D. Fructose-CFP	Veh: 71%-91%-77%	Veh: 81%-93%-91%	Veh: 79%-91%-77%	Veh: 81%-93%-91%
Acquisition Effects	SCH: 75%- 61%* - 59%*	SCH: 40%* - 46%* - 61%*	NTX: 88%-87%-75%	NTX: 72%-76%- 59%*

Significant drug effects are denoted in **bold** and with an asterisk (*).

The expression effects indicate percent CS+ intake following vehicle (0) and drug doses. The acquisition effects for fructose-CFP show the percent CS+ intake in vehicle (Veh)-, SCH23390 (SCH)- and naltrexone (NTX)-trained mice in Tests 1, 2 and 3.

The acquisition effects for sucrose-CFP (Dym et al., 2012) show the percent CS+ intake in vehicle (Veh)-, SCH23390 (SCH)- and naltrexone (NTX)-trained mice in Tests 1 and 2; a third test was not conducted.