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Double-dissociation of D1 and opioid receptor antagonism effects on the acquisition of sucrose-conditioned flavor preferences in BALB/c and SWR mice

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Abstract

Sugar appetite is influenced by unlearned attractions to sweet taste and learned responses to sugars' taste and post-ingestive actions. In rats, sugar-conditioned flavor preferences (CFP) are attenuated by dopamine D1 (SCH23390: SCH), but not opioid (naltrexone: NTX), receptor antagonism. Sucrose-CFP occurs in BALB/c and SWR inbred mice that differ in their suppressive effects of SCH and NTX on sucrose intake. The present study examined whether SCH and NTX altered expression of a previously learned sucrose-CFP and acquisition (learning) of sucrose-CFP in these strains. In Experiment 1, food-restricted mice were trained (10 one-bottle sessions) to drink a more-preferred flavored (e.g., cherry) 16% sucrose solution (CS+/Sucrose) on odd-numbered days, and a less-preferred flavored (e.g., grape) 0.05% saccharin solution (CS-/Saccharin) on even-numbered days. Two-bottle tests with the flavors mixed in 0.2% saccharin occurred 30 min following vehicle (Veh), SCH (50–800 nmol/kg) or NTX (1–5 mg/kg) assessing preference expression. CS+ preference expression in BALB/c and SWR mice following Veh were significantly reduced by SCH and NTX. In Experiment 2, separate groups of BALB/c and SWR mice received Veh, SCH (50 nmol/kg) or NTX (1 mg/kg) injections 30 min prior to daily one-bottle training sessions with the CS+/Sucrose and CS-/Saccharin solutions assessing preference acquisition. Subsequent two-bottle tests with the CS+ vs. CS- solutions were conducted without injections. CS+/Sucrose training intakes were reduced by SCH in both strains and by NTX in BALB/c mice. In the initial two-bottle test, sucrose-CFP acquisition was significantly reduced in BALB NTX (54%), but not in BALB SCH (77%) groups relative to the BALB Veh group (85%). In contrast, sucrose-CFP acquisition was significantly reduced in SWR SCH (61%), but not in SWR NTX (83%) groups relative to the SWR Veh group (86%). DA D1 and opioid receptor signaling modulate acquisition and/or expression of sucrose-CFP in mice with significant strain differences observed.

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Keywords

SCH23390; Naltrexone; Sugar conditioning; Genetic variance; Mouse

1. Introduction

Sweet taste preference is an important factor driving sugar intake in animals. This is documented by the significant differences in sucrose as well as non-nutritive sweetener intakes observed in inbred mouse strains with different alleles of the *Tas1r3* gene which results in “sensitive” and “subsensitive” forms of the T1R2/T1R3 sweet taste receptor (e.g., Lewis et al., 2005; Reed et al., 2004). However, variations in sweet taste sensitivity alone does not fully account for sugar intake and other genetic factors contribute to sugar appetite (Boughter and Bachmanov, 2007). Experiential factors can also greatly affect sugar preference and intake. For example, sucrose preference differences between a sweet sensitive inbred mouse strain (C57BL/6J, B6) and subsensitive strain (129P3/J, 129) disappeared after both strains had experience with dilute and concentrated sugar solutions (Sclafani, 2006). This experiential effect was attributed to a learned increase in sucrose preference due to the post-ingestive rewarding actions of the sugar. Supporting this interpretation, intragastric (IG) infusions of sucrose conditioned preferences for a flavored saccharin solution in both B6 and 129 strains (Sclafani & Glendinning, 2005).

Sugar-conditioned flavor preferences (CFP) are also produced by training animals to drink a sucrose solution containing one arbitrary flavor (the conditioned stimulus or CS+) and a less preferred saccharin solution or plain water containing a different flavor (the CS–) (Mehiel and Bolles, 1988; Myers and Hall, 2000). The learned preference for the CS+ flavor is revealed in a subsequent two-bottle choice test with the CS+ and CS– flavors presented in saccharin or plain water. Both the sweet taste and the post-ingestive nutritive actions of the sucrose contribute to the learned flavor preference (Capaldi, 1996; Sclafani, 1995).

Brain reward systems, including DA and opioid systems, are implicated in sugar appetite and preference both in rats and mice (Cooper, 2007; Dym et al., 2007, 2009; Levine et al., 2003), suggesting that these neurochemical systems may be involved in sugar-CFP as well. Studies with the Sprague-Dawley strain of rats revealed that DA receptor antagonism significantly impaired flavor-sugar conditioning (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b) whereas opioid receptor antagonism had little effect (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999), which was unexpected given opioid involvement in sugar appetite in general (Bodnar, 2004; Cooper, 2007; Levine et al., 2003). In a recent study with nine mouse strains, we reported strain differences in sucrose-CFP with SWR/J (SWR) and BALB/cJ (BALB) exhibiting the highest preferences (Pinhas et al., 2012). However, the role of DA and opioidergic systems in mediating sugar-CFP has not been investigated in mice.

There is evidence indicating that SWR and BALB strains of mice differ in the opioid control of sucrose intake. We recently reported that systemic treatment significantly with an opioid receptor antagonist, naltrexone (NTX) reduced 1-h sucrose intake to a lesser degree in SWR mice than in BALB mice (Dym et al., 2007). Furthermore, other data indicate that opioid-mediated reward processes may be compromised in SWR mice. In particular, SWR mice display significantly less morphine self-administration behavior as compared to 14 other inbred strains (Belknap et al., 1993) and display less morphine-induced conditioned place preferences (Gieryk et al., 2010; Solecki et al., 2009). In contrast, we found that SWR and BALB mice were similar in their suppression of sucrose intake in response to the D1 DA receptor antagonist SCH23390 (SCH) (Dym et al., 2009). Yet, BALB mice differed from 10

other inbred strains in their failure to self-administer cocaine, suggesting a sub-sensitivity in the DA reward system (Thomsen & Caine, 2011). However, SWR mice were not included in this study so it is not clear that BALB and SWR mice differ in this respect.

In the present study, therefore, we investigated the effects of SCH and NTX on sucrose-CFP in SWR and BALB mice. To this end, the effects of SCH (50–800 nmol/kg) and NTX (1–5 mg/kg) on the expression of a previously conditioned CS+ preference was evaluated by administering the drugs just prior to the two-bottle CS+ vs. CS– choice tests. Drug effects on the acquisition (learning) of this preference were evaluated by treating the animals with SCH (50 nmol/kg) or NTX (1 mg/kg) throughout one-bottle training, and subsequently conducting the two-bottle choice tests in the absence of drug treatments. In light of our previous studies on NTX- and SCH-induced suppression of sucrose intake in BALB and SWR mice (Dym et al., 2007, 2009), we predicted that NTX would be more effective in impairing sucrose-CFP in BALB than in SWR mice, but that SCH would be equally effective in attenuating sucrose-CFP in the two strains.

2. Materials and Methods

2.1. Subjects

Inbred BALB/ cJ and SWR/ J male mice (Jackson Laboratories, Bar Harbor, ME, 6 weeks of age) 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 entire study, and maintained on a 12 h light/ 12 h dark cycle (lights off at 2000 h) at a constant temperature of 22 °C. All animals were provided with chow (Lab Diet Mouse Chow 5015) and water *ad libitum* throughout the experiment, except when experimental testing was conducted. The experimental procedures 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.

2.2. Test solutions

The training solutions consisted of 16% sucrose (Domino Foods, Yonkers, NY) and 0.05% saccharin (Sigma Laboratories, St. Louis, MO) solutions flavored with 0.05% unsweetened grape or cherry Kool-Aid (Kraft Foods, White Plains, NY). For half of the mice in each strain, the CS+ flavor added to the sucrose solution was cherry and the CS– flavor added to the saccharin 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 a 0.2% saccharin solution as in our prior study (Pinhas et al., 2012). All testing took place in each mouse's home cage during the mid-light phase of the light:dark cycle. Two weeks before testing began, the mice were placed on a food restriction schedule that maintained their body weights at 85–90% of their *ad libitum* level. The mice were initially trained to drink unflavored 0.2% saccharin solution from a stainless steel sipper tube connected to a 10 ml plastic syringe (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. The limited food rations were given 1 h after each training and testing session.

2.3. Experiment 1: Expression procedure

A total of 31 BALB and 35 SWR inbred mice received ten one-bottle training sessions (1 h/ day) with 8 ml of the CS+/Sucrose 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 60-min 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 pair of two-bottle sessions, the mice were given either a subcutaneous (sc) or intraperitoneal (ip) vehicle (0.9% normal saline) injection as controls for the SCH and NTX groups respectively. Initially, 13 BALB and 11 SWR mice were injected sc with 200 and 800 nmol/kg doses of the D1 antagonist, SCH (Sigma Chemical Co.) prior to two test sessions each, with half tested with an ascending dose order and the remainder with a descending dose order. Given that these SCH doses potentially reduced overall CS intake (see Results), another cohort of 10 BALB and 9 SWR mice were injected twice with vehicle and a 50 nmol/kg SCH dose. SCH was mixed at concentrations of 5, 20 and 80 nmol/ml and administered at 10 ml/kg body weight as in a prior study (Dym et al., 2009). Eight BALB and 15 SWR mice were injected ip with 1 and 5 mg/kg doses of the general opioid antagonist, NTX (Sigma Chemical Co) with half tested with an ascending dose order and the remainder with a descending dose order. The drugs were mixed at concentrations of 0.1 and 0.5 mg/ml and administered at 10 ml/kg as in a prior study (Dym et al., 2007).

2.4. Experiment 2: Acquisition procedure

Twenty-one BALB and 21 SWR mice received 10 one-bottle training sessions (1 h/day) with the CS+/Sucrose solution presented on odd-numbered sessions, and the CS-/Saccharin solution presented on even-numbered sessions. Thirty min prior to each training session, eight BALB and eight SWR mice received either sc (n=4) or ip (n=4) vehicle injections, six BALB and six SWR mice received a sc injection of SCH (50 nmol/kg), and seven BALB and seven SWR mice received an ip injection of NTX (1 mg/kg). Following training, all mice were given four daily 2-bottle choice sessions (1 h/day) with access to the CS+ and CS- solutions; no drugs were administered prior to these sessions. The positions of the CS+ and CS- solutions were counterbalanced across testing sessions, and the results were analyzed as mean 60-min intakes during successive pairs of sessions (referred to as Tests 1 and 2).

2.5. Data analysis

In the expression studies, training intakes were averaged over the five CS+/Sucrose and five CS-/Saccharin sessions for each strain, and evaluated for differences. Intakes during the preference tests were averaged over the two sessions at each dose and evaluated with two-way analyses of variance (ANOVA, CS solution vs. Dose) for each strain and drug condition. Separate ANOVAs evaluated percent CS+ intakes as a function of drug dose for each strain. It should be noted that CS+ and CS- intakes following vehicle treatment in the two SCH cohorts failed to differ from each other, and these vehicle values were pooled.

In the acquisition studies, training intakes were averaged over the five CS+/Sucrose and five CS-/Saccharin sessions for the three (Veh, SCH, NTX) training groups and were analyzed with a two-way ANOVA (CS x Groups). Intakes during the preference tests were averaged over sessions 1 and 2 (Test 1) and 3 and 4 (Test 2) to control for side position effects. A three-way ANOVA compared the CS intakes of the vehicle, SCH and NTX groups (Group x CS x Test). Separate two-way ANOVAs evaluated percent CS+ intakes of the three groups. When main or interaction effects were found, Neuman-Kuels corrected comparisons ($p < 0.05$) detected significant effects. Drug-induced reductions in the acquisition or expression of sucrose-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 antagonist effects on the expression of sucrose-CFP in BALB and SWR mice

In BALB mice, the mean one-bottle training intake of the CS+/Sucrose solution significantly exceeded that of the CS-/saccharin solution (1.85 vs. 0.89 ml/1 h; $t(22)=12.70$, $p<0.0001$). Two-bottle tests, with both CS flavors presented in 0.2% saccharin, were then conducted following injections of SCH. Overall, BALB mice consumed significantly more CS+ than CS- ($F(1,55)=88.68$, $p<0.0001$) and there were significant drug dose ($F(3,55)=17.74$, $p<0.0001$) and CS x Dose interaction effects ($F(3,55)=18.07$, $p<0.0001$). CS+ intakes exceeded CS- intakes at the 0 (vehicle), 50 and 200, but not the 800, nmol/kg SCH doses (Figure 1, upper panel). BALB mice consumed significantly less CS+ at all SCH doses compared to vehicle; CS- intakes failed to differ as a function of SCH dose (Figure 1, upper panel). Total CS intakes were lower following the 50 (0.9 ml), 200 (0.7 ml) and 800 (0.4 ml) nmol/kg SCH doses relative to vehicle (1.6 ml). Percent CS+ intake was significantly reduced ($F(3,55)=4.95$, $p<0.004$) by the 200 (73%) and 800 (68%), but not the 50 (83%), nmol/kg SCH doses relative to the vehicle dose (85%) (Figure 1, upper panel).

In SWR mice, the mean one-bottle training intake of the CS+/Sucrose solution was significantly lower than that of the CS-/Saccharin solution (1.71 vs. 2.27 ml/1 h, $t(19)=3.54$, $p<0.002$). In the two-bottle preference tests, overall, the SWR mice consumed significantly more CS+ than CS- ($F(1,47)=33.33$, $p<0.0001$) and there were significant drug dose ($F(3,47)=23.94$, $p<0.0001$) and CS x Dose interaction effects ($F(3,47)=6.82$, $p<0.0007$). CS+ intakes significantly exceeded CS- intakes at the 0 (vehicle) and 50, but not at the 200 or 800 nmol/kg SCH doses (Figure 1, lower panel). SWR mice consumed significantly less CS+ at all SCH doses compared to vehicle; CS- intake significantly declined at the two higher SCH doses (Figure 1, lower panel). Total CS intakes significantly declined following the 50 (1.7 ml), 200 (0.8 ml) and 800 (0.6 ml) nmol/kg SCH doses relative to vehicle (2.6 ml). Although the percent CS+ intakes dose-dependently declined following the 0 (75%), 50 (71%), 200 (66%) and 800 (61%) nmol/kg SCH doses in SWR mice, this effect failed to achieve significance ($F(3,47)=1.69$, ns) (Figure 1, lower panel).

3.2. Opioid antagonist effects on the expression of sucrose-CFP in BALB and SWR mice

In BALB mice, the mean one-bottle training intake of the CS+/Sucrose solution significantly exceeded that of the CS-/Saccharin solution (1.76 vs. 0.78 ml/1 h, $t(7)=8.16$, $p<0.0001$). In the two-bottle preference tests following NTX treatment, overall, BALB mice consumed significantly more CS+ than CS- ($F(1,21)=30.77$, $p<0.0001$), and the drug dose and CS x Dose interaction effects were not significant. CS+ intakes exceeded CS- intakes at the 0 (vehicle) and 1, but not the 5 mg/kg NTX doses (Figure 2, upper panel). Total CS intakes failed to change following vehicle (1.7 ml) and the 1 (1.4 ml) and 5 (1.2 ml) mg/kg NTX doses. Although percent CS+ intakes dose-dependently declined following the 0 (74%), 1 (76%) and 5 (63%) mg/kg NTX doses, this effect failed to achieve significance ($F(2,23)=1.16$, ns) (Figure 2, upper panel).

In SWR mice, the mean one-bottle training intake of the CS+/Sucrose and CS-/Saccharin solutions did not significantly differ (1.55 vs. 1.40 ml/1 h). In the two-bottle preference tests, overall, the SWR mice consumed significantly more CS+ than CS- ($F(1,42)=40.59$, $p<0.0001$), and there were significant drug dose ($F(2,42)=4.42$, $p<0.018$) and CS x Dose interaction effects ($F(2,42)=5.47$, $p<0.008$). CS+ intakes significantly exceeded CS- intakes at all three doses (Figure 2, lower panel). SWR mice consumed significantly less CS+ at the 1 and 5 mg/kg NTX doses compared to vehicle; CS- intake did not differ across NTX doses

(Figure 2, lower panel). Total CS intakes significantly declined following the 1 (1.9 ml) and 50 (1.5 ml) mg/kg NTX doses relative to vehicle (2.5 ml). Percent CS+ intakes were significantly lower following the 1 (63%) and 5 (63%) mg/kg NTX doses relative to vehicle (78%) in SWR mice ($F(2,42) = 3.96$, $p < 0.027$).

3.3. DA D1 and opioid antagonist effects on the acquisition of sucrose-CFP in BALB mice

Overall, the BALB mice groups treated with Veh, SCH or NTX during one-bottle training consumed significantly more CS+/Sucrose than CS-/Saccharin (1.29 vs. 0.69 ml/1 h, $F(1,18) = 133.44$, $p < 0.0001$). Total training intakes were significantly lower in the SCH (1.1 ml/1 hr) and NTX (1.9 ml/1 h) groups relative to the Veh group (2.7 ml) ($F(2,18) = 39.02$, $p < 0.0001$); the Group x CS interaction approached, but did not achieve significance ($F(2,18) = 2.78$, $p = 0.089$) (Figure 3, upper panel). In the two-bottle preference tests conducted without drug injections, overall, the BALB mice consumed significantly more CS+ than CS- ($F(1,18) = 43.20$, $p < 0.0001$); there were no other significant group, test, or interaction effects. In the preference tests, CS+ intake was significantly higher than corresponding CS- intake in the two tests in Veh and SCH groups. In the NTX group, CS+ and CS- intakes failed to differ in Test 1, but CS+ intake was significantly higher in Test 2 (Figure 3, lower panel). Overall, percent CS+ preference was significantly lower ($F(2,18) = 4.05$, $p < 0.035$) in the NTX group (60%) than in the Veh (81%) and SCH (79%) groups; there were no significant test or Test x Group interaction on this measure.

3.4. DA D1 and opioid antagonist effects on the acquisition of sucrose-CFP in SWR mice

Overall, the SWR groups treated with Veh, SCH or NTX during one-bottle training consumed significantly less CS+/Sucrose than CS-/Saccharin (1.35 vs. 2.07 ml/1 h, $F(1,18) = 31.15$, $p < 0.0001$; Figure 4, upper panel). There were also significant group ($F(2,18) = 21.19$, $p < 0.0001$) and Group x CS interaction effects ($F(2,18) = 11.72$, $p < 0.0001$). Total CS intake was significantly lower in the SCH group than the Veh group; intakes in the NTX and Veh groups failed to differ. In addition, CS-/Saccharin intake was significantly greater than CS+/Sucrose intake in the NTX and Veh but not the SCH groups (Figure 4, upper panel). In the two-bottle preference tests, overall, SWR mice consumed significantly more CS+ than CS- ($F(1,18) = 126.72$, $p < 0.0001$) and there were Test ($F(1,18) = 8.21$, $p < 0.01$), Group x CS ($F(2,18) = 5.62$, $p < 0.013$) and Test x CS ($F(1,18) = 5.39$, $p < 0.032$) interaction effects, but no Group effect. In the preference tests, CS+ intakes significantly exceeded CS- intakes in both tests in the Veh and NTX SWR groups. In SCH SWR mice, CS+ and CS- intakes failed to differ in Test 1, but CS+ intake was significantly higher in Test 2 (Figure 4, lower panel).

Overall, percent CS+ intake was significantly lower ($F(2,18) = 4.90$, $p < 0.02$) in the SCH group (68%) than in the NTX (84%) and Veh (88%) groups (Figure 4, lower panel). Overall, the CS+ preferences following Veh treatment in the expression experiments and following Veh training in the acquisition experiments did not significantly differ ($t(38,42) = 1.08$, ns) between the BALB ($n = 39$, 81.9% (± 2.0 SEM)) and SWR ($n = 43$, 78.6% (± 2.4 SEM)) strains.

4. Discussion

In this study, we explored the role of DA D1-like receptor and opioid receptor signaling in BALB and SWR strains of mice in flavor preference learning induced by the ingestion of sucrose. These strains were selected based on their similar robust sucrose-CFP in a survey of nine strains (Pinhas et al., 2012) and their differential NTX-induced suppression of sucrose intake (Dym et al., 2007). The results revealed that antagonism of D1-like receptors impaired the acquisition of sucrose-CFP in SWR, but not BALB mice. In contrast, antagonism of opioid receptors disrupted the acquisition of sucrose-CFP in BALB, but not

SWR mice. The expression of sucrose-CFP was reduced by SCH and NTX in both strains. These findings demonstrate that DA and opioid reward systems can independently contribute to the establishment of sucrose-CFP in different inbred mouse strains.

Consistent with prior results (Pinhas et al., 2012), the BALB and SWR mice learned preferences for the sucrose-paired CS+ flavor that were of similar magnitude (82% and 79%, respectively). The strains were also comparable in their training intakes of the CS+ solution, but differed in that the BALB mice consumed significantly less CS- than CS+ solutions, whereas the SWR mice consumed as much or more CS- than CS+ during training. We previously observed similar training intake differences in BALB and SWR strains (Pinhas et al., 2012). We speculated that the higher intake of the saccharin-sweetened CS- by SWR mice was due to their having the sensitive form of the sweet taste receptor which increases saccharin preference and intake relative to that observed in mice with the sweet sub-sensitive receptor including BALB mice (Reed et al., 2004). SWR mice also drink more sucrose than BALB mice in 24-h tests (Lewis et al., 2005), but interestingly not in the 1-h training sessions in the present and previous (Pinhas et al., 2012) studies.

4.1 DA D1 signaling and modulation of sucrose-CFP learning

When treated with SCH prior to the two-bottle choice tests (expression experiment), both strains reduced their CS intakes and CS+ preferences. The strains differed only in that the SWR mice failed to prefer the CS+ at both 200 and 800 nmol/kg doses, whereas the BALB mice lost their preference only at the 800 nmol/kg dose. In the acquisition experiment, SCH treatment during training prevented the SWR mice from displaying a significant CS+ preference in the first test (61% in SCH mice vs. 86% in Veh mice), but a significant, albeit attenuated CS+ preference emerged in the second test (74% vs. 89% in Veh mice). The initial absence and subsequent recovery of the sucrose-CFP observed in the SWR SCH group was unexpected. One would expect if anything, that the CS+ preference would decline with repeated testing due to “extinction” given that the CS+ flavor was no longer paired with sucrose. Note, however, that the SWR Veh mice tended to increase their CS+ intake and preference in Test 2 and we previously observed persistent CS+ preferences over three tests in SWR mice (Pinhas et al., 2012). One possible explanation for the low Test 1 CS+ preference displayed by the SWR SCH mice was a drug state dependency effect that dissipated in Test 2. According to this view, which requires further testing, SWR SCH mice may display a stronger CS+ preference if they were treated with SCH prior to Test 1. It should also be noted that the suppressed training intakes of the SWR SCH may have contributed to their reduced CS+ preference displayed in Tests 1 and 2.

In contrast to SWR mice, SCH failed to reduce the acquisition of the CS+ preference in the BALB mice relative to BALB vehicle controls in Test 1 (77% vs. 85%) and Test 2 (80% vs. 77%). It is possible that higher SCH doses administered during training would have blocked CS+ preference learning in the BALB mice. However, higher training SCH doses may reduce training intakes to low levels that precluded learning. This is suggested by the low CS intakes produced by the 200 and 800 nmol/kg SCH doses in the expression study.

Taken together, the findings indicate that SCH impairs the expression and acquisition of a sucrose-CFP more in SWR mice than in BALB mice. The pattern of these results differs from SCH-induced reductions in sucrose intake in these strains. Our laboratory (Dym et al., 2009) previously found that SCH produced similar dose-dependent reductions in 1-h sucrose intake in the two strains following the 200 (BALB: 17% inhibition; SWR: 6% inhibition; $t=0.50$, ns) and 800 (BALB: 74%; SWR: 61% inhibition; $t=0.91$, ns) nmol/kg doses employed in the present study. Thus, the strain differences in the SCH effect on sucrose-CFP are not readily explained by the drug effects on sucrose intake per se, but may be due specifically to drug effects on preference learning. However, in a parallel study (Dym et al., 2010)

examining DA D1 antagonist effects on fat (Intralipid) intake, SCH reduced Intralipid intake to a far greater degree in SWR mice than in BALB mice following the 200 (BALB: 26% increase; SWR: 44% inhibition; $t = 2.80$, $p < 0.022$) and 800 (BALB: 10% increase; SWR: 68% inhibition; $t = 3.16$, $p < 0.009$) nmol/kg doses. Therefore, it will be of interest to compare SCH drug effects in SWR and BALB mice using other preference learning paradigms such as fat-CFP.

In prior rat experiments, we investigated the effect of SCH on flavor conditioning using different training paradigms to separate the conditioning effects of sweet taste vs. post-ingestive nutrient actions. Overall systemic SCH administration at high doses prior to preference testing reduced the expression of the CS+ preference in rats trained with real-fed fructose, sham-fed sucrose, or IG sucrose infusion (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000a, 2000b). This is consistent with the reduced expression of the sucrose-conditioned CS+ preference produced by SCH treatment in the BALB and SWR mice. In addition, systemic SCH treatment during training attenuated or blocked the acquisition of sugar-CFP in rats trained with these three different paradigms (Azzara et al., 2001; Baker et al., 2003; Yu et al., 2000b). These effects are consistent with the failure of the SWR SCH mice to display a significant CS+ preference in Test 1 but conflict with their recovery of a CS+ preference in Test 2 and the persistent CS+ preference displayed by BALB SCH mice. As mentioned above, a higher SCH training dose may more profoundly impair sucrose conditioning in mice. Alternatively, the different training procedures used in the rat and mouse experiments may contribute to the discrepant results. In particular, whereas the mouse in the present study were exposed to both the sweet taste and positive post-ingestive actions of sugars, the rats in the prior studies experienced only sweet taste or post-ingestive sugar rewards.

As reviewed elsewhere (Sclafani et al., 2011), there is extensive evidence that central DA circuits are involved in food reward processing. Of particular relevance to sugar conditioning studies are reports that the sweet taste as well as the post-ingestive actions of sugar promote DA release in the nucleus accumbens (NAc) (Hajnal et al., 2004; Ren et al., 2010). Furthermore, local SCH microinjections into the NAc during training blocked or attenuated sugar-conditioned preferences in rats (Bernal et al., 2008; Touzani et al., 2008) and similar effects were observed in the amygdala and medial prefrontal cortex regions which receive projections of the DA reward system (Bernal et al., 2009; Malkusz et al., 2012; Touzani et al., 2009, 2010). Thus, differences in the response to D1-like receptor antagonism in BALB and SWR mice may be related to differences in the sensitivity to SCH or a defect (affinity, number) in the D1-like receptors in the BALB mice. To the best of our knowledge, there are no published studies comparing the D1 receptor ligand binding in these two strains, which warrant further investigation.

4.2 Opioid signaling and modulation of sucrose-CFP learning

In the expression experiments, when BALB mice were treated with NTX prior to preference testing, the high dose significantly reduced CS+ intake to a level which no longer significantly exceeded CS- intake, and non-significantly reduced the preference from 74% to 63%. SWR mice were more influenced by NTX treatment; their total CS intake and percent CS+ preference in the expression study were significantly reduced following both NTX doses compared to vehicle treatment. On the other hand, in the acquisition experiment, BALB mice were more impaired than SWR mice by NTX treatment during training. The BALB NTX mice failed to prefer the CS+ (54%) in Test 1 although they displayed a significant although reduced preference in Test 2 (66% vs. 77% in Veh mice). In contrast, NTX failed to impair the acquisition of the sucrose-CFP in the SWR mice as revealed by their robust CS+ preferences in both tests compared to the Veh mice (83% vs. 86% and 85% vs. 89% in Tests 1 and 2, respectively). It is possible that a higher NTX training dose may

impair the acquisition of a sucrose-CFP in SWR mice. However, higher training NTX doses may reduce training intakes to low levels that precluded learning. As with the results obtained with the SWR SCH mice, the recovery of a significant CS+ preference by the BALB NTX mice in Test 2 was unexpected. In this case, the BALB Veh showed a slight reduction not increase in CS+ preference from Test 1 to 2. Further work is needed to determine if the low Test 1 CS+ preference displayed by the BALB NTX mice was a drug state dependency effect that dissipated in Test 2.

We previously observed that NTX significantly reduced 1-h sucrose intake to a greater degree in BALB mice than SWR mice (Dym et al., 2007), an effect consistent with the NTX effects on CS training intakes observed in the present study. SWR mice appear to show attenuated opioid-mediated responses to rewards. These mice displayed significantly less morphine self-administration behavior as compared to 14 other inbred strains (Belknap et al., 1993) and were impaired in morphine-induced conditioned place preferences (Gieryk et al., 2010; Solecki et al., 2009). These findings along with our results suggest that SWR mice have a hyposensitive opioid reward system, perhaps due to reductions in the number and/or affinity of opiate receptors in the brain areas involved in processing reward and reward-related learning.

In contrast to the present results, our earlier studies with Sprague-Dawley rats (outbred strain) revealed little or no effect of NTX treatment on the expression or acquisition of sugar-CFP. This was true for rats trained with real-fed fructose, sham-fed sucrose, or IG sucrose infusions (Azzara et al., 2000; Baker et al., 2004; Yu et al., 1999). Given the extensive literature implicating central opioid receptors in sweet taste reward (see reviews: Bodnar, 2004; Cooper, 2007; Levine et al., 1995, 2003), it was surprising that NTX treatment had virtually no effect on sugar-CFP in Sprague-Dawley rats. The possibility that other strains of rats might be sensitive to the antagonistic effect of opiate receptors on CFP remains an open question. A recent study in rats reported that NTX treatment did not differentially alter sucrose motivation in rats selectively bred to drink high or low amounts of saccharin but drug effects on sucrose-CFP were not examined (Gosnell et al., 2010). As noted above, the different training procedures used in the rat and mouse experiments may contribute to the discrepant results obtained with NTX.

In summary, the present findings demonstrate a double-dissociation in the effects of DA and opioid receptor antagonism on the acquisition of sucrose-CFP in two-inbred mouse strains; SWR and BALB. The two strains were selected for study because they displayed comparable strong sucrose-CFP despite differing in their sweet taste sensitivity. As in rats, SCH treatment attenuated the expression of the sucrose-CFP in both strains but suppressed preference learning in only the SWR mice. Unlike rats, however, the acquisition of sucrose-CFP was impaired by NTX in BALB mice, which recommends this strain for further study of opioid mediation of food-related learning. These findings indicate clearly that both the DA and opioidergic reward systems can equally and independently contribute to the establishment of sucrose-CFP in rodents and that caution must be exerted in interpreting reward-related learning findings in rat studies using the Sprague-Dawley strain.

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Research Highlights

- Dopamine D1 antagonism reduced sucrose preference expression in BALB and SWR mice.
- Opioid antagonism reduced sucrose preference expression in BALB and SWR mice.
- Only opioid antagonism reduced sucrose preference acquisition in BALB mice.
- Only dopamine D1 antagonism reduced sucrose preference acquisition in SWR mice.
- Murine genetic background influences the pharmacology of sucrose preferences.

Figure 1. (Expression Procedure). Intakes (mean $\pm$ SEM, g/1 h) of CS+ and CS- solutions in two-bottle tests in BALB (upper panel) and SWR (lower panel) inbred mice receiving systemic injections of the DA D1-like antagonist, SCH23390 at doses of 0, 50, 200 and 800 nmol/kg 30 min prior to testing. Significant differences in this and the next figure 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.

(Expression Procedure). Intakes (mean $\pm$ SEM, g/1 h) of CS+ and CS- solutions in two-bottle tests in BALB (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.

Figure 3. (Acquisition Procedure). Upper Panel: Training intakes (mean +SEM, g/1 h) of CS+/Sucrose and CS-/Saccharin solutions in BALB mice pretreated 30 min earlier with vehicle (Veh), SCH23390 at a dose of 50 nmol/kg (SCH) or naltrexone at a dose of 1 mg/kg (NTX). Significant differences in this and the next figure are denoted between CS+/Sucrose and CS-/Saccharin intakes within each group (*) as are significant differences in CS+/Sucrose or CS-/Saccharin intakes following SCH or NTX relative to corresponding Veh (+). Lower Panel: Testing intakes (mean +SEM, g/1 h) of CS+ and CS- solutions during two-bottle Tests 1 and 2 in BALB mice receiving Veh, SCH or NTX during training. Numbers atop bars represent the mean percent intakes of CS+ (%CS+). Significant differences in this and the next figure are denoted between CS+ and CS- intake and for %CS+ intake within each test (*).

Figure 4.

(Acquisition Procedure). Upper Panel: Training intakes (mean $\pm$ SEM, g/1 h) of CS+/Sucrose and CS-/Saccharin solutions in SWR mice pretreated 30 min earlier with vehicle (Veh), SCH23390 at a dose of 50 nmol/kg (SCH) or naltrexone at a dose of 1 mg/kg (NTX). Lower Panel: Testing intakes (mean $\pm$ SEM, g/60 min) of CS+ and CS- solutions during two-bottle Tests 1 and 2 in SWR mice receiving Veh, SCH or NTX during training. Numbers atop bars represent the mean percent intakes of CS+ (%CS+). The Y-axis has been modified to accommodate the increased intake of this strain in this paradigm.