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Strain differences in muscarinic cholinergic receptor antagonism of fat intake and acquisition and expression of fat-conditioned flavor preferences in male BALB/c, C57BL/6 and SWR mice

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ABSTRACT

Murine strain differences occur for both intakes of and preferences for sugars and fats. Previous studies demonstrated that the muscarinic cholinergic receptor antagonist, scopolamine (SCOP) more potently reduced sucrose and saccharin intakes in inbred C57BL/6 and BALB/c than SWR mice, sucrose-conditioned flavor preferences (CFP) expression in BALB/c, but not C57BL/6 or SWR mice, and sucrose-CFP acquisition in BALB/c relative to SWR and C57BL/6 mice. Although fat intake and fat-CFP are observed in all three strains, strain-specific effects were previously observed following dopamine D1, opiate and NMDA receptor antagonism of sweet and fat intake and CFP. The present study investigated whether muscarinic receptor antagonism differentially affected fat (Intralipid) intake and preferences in these strains by examining whether SCOP altered fat (Intralipid) intake and fat-CFP expression and acquisition in BALB/c, C57BL/6 and SWR mice. SCOP (0.1–10 mg/kg) significantly reduced Intralipid (5%) intake in all three strains across 2 h. In fat-CFP expression experiments, food-restricted mice consumed one flavored (conditioned stimulus (CS)+, 5 sessions) Intralipid (5%) solution and a differently-flavored (CS−, 5 sessions) Intralipid (0.5%) solution. Two-bottle CS choice tests with the two flavors mixed in 0.5% Intralipid occurred following vehicle and two SCOP doses (1, 5 mg/kg). SCOP elicited small, but significant reductions in fat-CFP expression in BALB/c and C57BL/6, but not SWR mice. In fat-CFP acquisition experiments, separate groups of BALB/c, C57BL/6 and SWR mice were treated prior to the ten acquisition training sessions with vehicle or two SCOP (2.5, 5 mg/kg) doses followed by six two-bottle choice tests without injections. SCOP eliminated fat-CFP acquisition in all three strains. Thus, muscarinic receptor signaling mediates learning, and to a lesser degree maintenance of fat-CFP while maximally inhibiting fat intake in the three strains.

1. Introduction

Fats and sugars contribute to the palatability of foods through both their inherent hedonic properties and the learning of preferences in rodents (see reviews: Bodnar, 2018; Bodnar et al., 2013). Acetylcholine (Ach), and particularly its muscarinic cholinergic receptor, has been implicated in the mediation of food intake in rats, particularly sweet and fat intake (Perry et al., 2009; Pratt and Blackstone, 2009; Pratt et al., 2007; Will et al., 2006). Ach is also involved in the mediation of rat sweet- and fat-CFP (Rotella et al., 2015, 2016), a form of classical conditioning in which a particular flavor (the conditioned stimulus, CS+) is associated with the oral and/or post-oral properties of nutrients (the unconditioned stimulus, US). Pharmacological control of sweet- and fat-CFP can affect both initial learning (acquisition) and continued maintenance (expression) of the preference. In rats, expression of sugar (fructose)- and fat (corn oil)-CFP was significantly, though minimally reduced by systemic administration of muscarinic (scopolamine (SCOP)) and nicotinic (mecamylamine) cholinergic receptor antagonists (Rotella et al., 2015, 2016). In contrast, sugar- and fat-CFP acquisition was eliminated by SCOP, but not mecamylamine in rats (Rotella et al., 2015, 2016), indicating that the muscarinic cholinergic receptor system is essential for acquisition (learning) of both fat-

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induced and sugar (fructose)-induced CFP in rats.

Evaluation of inbred murine strain differences provide important sources of information regarding the genetic control of many aspects of ingestive behavior, including the presence of genetic variance in ingestive responses and identification of mouse strains with divergent sensitivities for quantitative trait loci (QTLs) analyses to localize chromosomal regions, and ultimately genes, critically involved in such differences (see reviews: Bodnar et al., 2013; Reed et al., 1997; West and York, 1998). Three inbred murine strains (BALB/c, C57BL/6 and SWR) were chosen for study because they displayed among the strongest sensitivities to Intralipid intake in a murine strain survey (Lewis et al., 2007), and each elicit strong Intralipid-CFP (Kraft et al., 2013). A role for murine genetic variance was recently observed in SCOP's ability to decrease sweet intake with potent reductions of sucrose and saccharin intake in C57BL/6 and BALB/c mice, but only transient (sucrose) and less-potent (saccharin) reductions were noted in SWR mice (Bourie et al., 2017). SCOP significantly reduced the magnitude of the expression of sucrose-CFP in BALB/c, but not C57BL/6 or SWR mice (Iskhakov et al., 2018). Further, SCOP dose-dependently reduced (1 mg/kg) and eliminated (2.5 mg/kg) the acquisition of sucrose-CFP in BALB/c mice, and reduced the magnitude of acquisition of sucrose-CFP in SWR mice. In contrast, SCOP failed to affect the acquisition of sucrose-CFP in C57BL/6 mice (Iskhakov et al., 2018), demonstrating that muscarinic cholinergic receptor signaling is essential for the learning of sucrose-CFP in BALB/c mice, to a lesser degree in SWR mice, but not in C57BL/6 mice.

It is not known whether the same pattern of murine strain-specific effects is observed in the muscarinic cholinergic receptor mediation of fat intake or fat-CFP. Our laboratory used an emulsified fat solution, Intralipid, to deliver fat through an oral, liquid route in an attempt to make it as comparable as sucrose and saccharin intake. Previous pharmacological studies that are detailed in the Discussion section indicate that important differences in the profiles of intake and preference responses emerge as functions of strain (BALB/c vs. C57BL/6 vs. SWR), type of test (intake per se, CFP expression, CFP acquisition) and neurochemical receptor under study (dopamine D1, opioid, NMDA). Therefore, the present study examined whether muscarinic cholinergic receptor antagonism with SCOP differentially altered fat (Intralipid) intake as well as the acquisition and expression of fat (Intralipid)-CFP in BALB/c, SWR and C57BL/6 mice.

2. Experiment 1 Scopolamine effects upon Intralipid intake in SWR, C57BL/6 and BALB/c mice

2.1. Materials and methods

2.1.1. Subjects

Inbred BALB/c (Stock #000651), C57BL/6 (Stock #000664) and SWR (Stock #000689) male mice (Jackson Laboratories, Bar Harbor, ME, 6 weeks of age, all strains ~25 g at arrival) were acclimated to the Queens College vivarium for one week in group (5 per cage) housing. The age of the animals was consistent with our previous behavioral and pharmacological studies of fat intake and fat-CFP (Dym et al., 2010; Kraft et al., 2013, 2017; Lewis et al., 2007). 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 for two more weeks. All experimental tests commenced around 1000 h, or 2 h into the animal's light cycle. Throughout the experiment, all animals were provided with chow (Lab Diet Mouse Chow 5015) and water ad libitum, except when testing procedures were being performed. The procedures used for both experiments were approved by the Queens College Institutional Animal Care and Use Committee. All subjects and experimental procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.1.2. Testing apparatus

Intralipid, purchased from Baxter Laboratories in a 20% solution, contains 20% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerin, and water with sodium hydroxide added to adjust the pH so that the final product pH is 8. The 20% Intralipid solution was diluted to the tested 5% and 0.5% concentrations by adding water. Visual measurements of Intralipid intake (± 0.1 ml) were accomplished using a retrofitted testing sipper tube made of a leur slip tip syringe (10 ml, 0.2 ml gradations, Pharmaseal Laboratories, Glendale, CA), a rubber cork, a straight sipper tube (63 mm in length, 8 mm in width, Lab Products, Seaford, DE), and a silicone sealant (All-Glass Aquarium Co., Inc., Franklin, WI) (Bourie et al., 2017; Dym et al., 2010; Kraft et al., 2013). After removing and drilling a hole into the tip of the syringe, the sealant secured the cork to the sipper tube preventing leakage. The sipper tubes were affixed to the stainless steel top of the cage with a metal spring (100 mm) with clips at each end of the metal spring secured to the top of the cage in order to allow for accurate readings of the meniscus.

2.1.3. Intralipid intake procedure

Prior to the testing procedure, chow and water were removed from each animal's cage at 3–7 h into the light cycle. Each animal received approximately 8 ml of a 5% Intralipid solution (Baxter Laboratories) in a sipper tube attached to their home cage for 2 h in order to measure their baseline intake. Approximately 2 ml of air was left in the sipper tube to permit for easy access and licking of the fat solution. Intralipid intake was measured after 5, 15, 30, 45, 60, 90, and 120 min by reading the meniscus of the solution along the gradations consistent with previous studies (Bourie et al., 2017; Dym et al., 2010; Kraft et al., 2013). After the last reading was taken for each animal, the sipper tubes were immediately removed from the cage, and food and water were returned approximately 30 min thereafter. This procedure was repeated daily until all animals reached the minimum criterion of drinking at least 1 ml of the solution over three consecutive days. This was done so that any inhibitory effects of the antagonists could be adequately assessed, and not subject to “floor effects”.

2.1.4. Muscarinic receptor antagonist effects upon Intralipid intake in BALB/c, C57BL/6 and SWR mice

Following this initial training period, each of 10 BALB/c, 10 C57BL/6 and 10 SWR mice received an intraperitoneal (ip) vehicle injection (0.3 ml 0.9% normal saline/30 g body weight, 10 ml/kg) 30 min prior to the presentation of the 5% Intralipid solution with intake was measured as above for 2 h. Each mouse was then tested at minimal 72 h intervals across the same time course procedure following i.p. injection of SCOP (Sigma Chemical Co., St. Louis, MO) at total doses of 0.1, 1.0, 2.5, 5.0 and 10 mg/kg, a dose range previously evaluated in rats (Rotella et al., 2015) and these inbred mouse strains (Bourie et al., 2017). SCOP was mixed at concentrations of 0.01, 0.1, 0.25, 0.5 and 1 mg/ml of 0.9% normal saline, and administered i.p. at 10 ml/kg body weight. Half of the animals of each strain, matched for vehicle Intralipid intake, received an ascending series of SCOP doses, whereas the other half received a descending series of SCOP doses as done previously (Bourie et al., 2017). A minimum of 72 h elapsed between injections to minimize carry-over effects as done previously (Bourie et al., 2017; Dym et al., 2010; Kraft et al., 2013).

2.1.5. Statistics

An initial analysis revealed that significant differences in cumulative Intralipid intake following vehicle in BALB/c C57BL/6 and SWR strains failed to occur among strains ($F(2,18) = 1.23$), across the time course ($F(6,54) = 2.12$) or for the interaction between strains and times ($F(12,108) = 0.83$). Therefore, as performed in previous pharmacological analyses (Bourie et al., 2017; Dym et al., 2010; Kraft et al., 2013), separate two-way repeated-measures analyses of variance were performed on cumulative Intralipid intake of BALB/c, C57BL/6 or SWR mice with antagonist drug dose as one within-subject variable, and the

seven intake time points as the second within-subject variable. Tukey comparisons ($p < 0.05$) evaluated significant drug effects within groups. Finally, post-drug intake difference scores were also calculated by subtracting Intralipid intake 60 min following each antagonist dose condition from its corresponding vehicle intake for each animal in each strain. Then linear regression analyses were performed for each strain with the log dose of the antagonist as the independent variable and the difference scores for each mouse in each strain as the dependent variable to determine the dose that would inhibit Intralipid intake by 40% (ID_{40}) (Bourie et al., 2017). A criterion of a dose necessary to produce a 40% inhibition of intake was chosen relative to the more typical ID_{50} criterion for the following reasons. First, in all cases, a 40% inhibition in intake by the antagonist was always significantly different from vehicle. Second, the use of the ID_{40} relative to the ID_{50} yielded effective doses that were invariably within and rarely outside of the actual antagonist dose ranges used, resulting in interpolated as compared to extrapolated data.

2.2. Results

2.2.1. Muscarinic receptor antagonism and Intralipid intake across inbred mouse strains

Significant differences in Intralipid intake in BALB/c mice were observed among SCOP doses ($F(5,54) = 9.95$, $p = 0.0001$), across test times ($F(6,324) = 82.85$, $p < 0.0001$) and for the interaction between doses and times ($F(30,324) = 4.03$, $p = 0.0001$). SCOP significantly reduced Intralipid intake in BALB/c mice following the 0.1 (15–120 min), 1 (15–120 min), 2.5 (15–120 min), 5 (15–120 min) and 10 (5–120 min) mg/kg doses (Fig. 1A). Significant differences in Intralipid intake in C57BL/6 mice were observed among SCOP doses ($F(5,54) = 9.21$, $p < 0.0001$), across test times ($F(6,324) = 90.59$, $p \leq 0.0001$) and for the interaction between doses and times ($F(30,324) = 4.58$, $p = 0.0001$). SCOP significantly reduced Intralipid intake in C57BL/6 mice across the entire time course and SCOP dose range (Fig. 1B). Significant differences in Intralipid intake in SWR mice were observed among SCOP doses ($F(5,54) = 20.55$, $p = 0.0001$), across test times ($F(6,324) = 30.52$, $p = 0.0001$) and for the interaction between doses and times ($F(30,324) = 3.44$, $p = 0.0001$). SCOP significantly reduced Intralipid intake in SWR mice across the entire time course and SCOP dose range (Fig. 1C). SCOP produced potent inhibitory ($ID_{40} < 0.1$ mg/kg) effects upon Intralipid intake in all three strains.

3. Experiment 2 Scopolamine effects upon Intralipid-CFP in SWR, C57BL/6 and BALB/c mice

3.1. Materials and methods

3.1.1. Subjects and initial training procedures

Naïve inbred BALB/c, C57BL/6 and SWR male mice were initially acclimated to the Queens College vivarium, then housed individually and maintained with chow and water as described in Experiment 1. Two weeks before CFP training began, the mice were placed on a food restriction schedule in which 2–3 g of chow was placed in their cages daily with water available ad libitum. Animals were placed on this schedule to insure short-latency sampling of the solutions during initial training and subsequent testing, and mice stabilize at 85–90% within 8–10 days of being placed on this regimen. During initial training and subsequent CFP expression and acquisition testing, the 2–3 g food ration was be provided to the mice 1 h after the completion of the test. The mice were weighed just prior to the onset of food-restriction, and their body weights were maintained at 85–90% of ad libitum levels during training/testing. All experimental tests commenced around 1000 h, or 2 h into the animal's light cycle.

3.1.2. Initial training

While food-restricted, the mice were trained 1 h/day to drink an unflavored 0.2% saccharin solution (Sigma-Aldrich Company, St. Louis MO) from a stainless steel sipper tube connected to a 10 ml plastic syringe (Kraft et al., 2017). This training procedure was repeated daily until all mice sampled the sipper tubes with short (< 1 min) latency, typically within three days.

3.1.3. Intralipid-CFP expression procedure

Two Intralipid concentrations (5% and 0.5%) capable of eliciting vigorous intake were utilized in the Intralipid-CFP paradigm each flavored with 0.05% grape or cherry Kool-Aid (Kraft Foods, White Plains, NY) as in previous studies (Lewis et al., 2007; Kraft et al., 2013, 2017). Half of the mice for each strain had a cherry CS+ flavor added to the more concentrated Intralipid (5%: CS+/I) solution, and a grape CS− flavor added to the less concentrated Intralipid (0.5%: CS−/i) solution; the flavor-sweetener pairs were reversed for the remaining animals. In the 2-bottle preference tests, the CS+ and CS− flavors were each presented in a 0.5% Intralipid concentrated solution as in previous studies (Kraft et al., 2013, 2017). Training and testing took place in the home cages during the mid-portion of the light phase. The limited food rations were given 1 h after each training and testing session. The mice received ten one-bottle training sessions (1 h/day) with 8 ml of the CS+/I solution presented on odd-numbered days, and 8 ml of the CS−/i solution presented on even-numbered days. On days 9 and 10, the mice also had access to a second sipper tube containing water, familiarizing 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 two sipper tubes systematically 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 2-bottle choice test sessions (1 h/day) with the CS+ and CS− solutions. Thirty min prior to the first two sessions, an

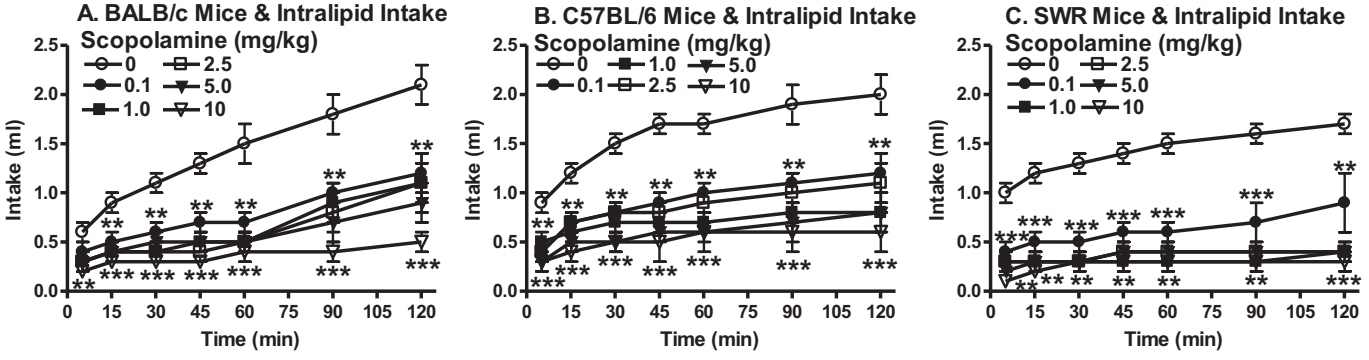

Fig. 1. Alterations (mean, ± SEM) in Intralipid (5%) intake following the five doses of the muscarinic cholinergic receptor antagonist, scopolamine in BALB/c (Panel A), C57BL/6 (Panel B) and SWR (Panel C) mice. Significant differences in intake following scopolamine relative to corresponding vehicle intake are denoted (*).

intraperitoneal (i.p.) vehicle (Veh: 0.9% normal saline) injection was administered to 10 BALB/c, 10 C57BL/6 and 10 SWR mice. Then on the subsequent four test days, the mice received two choice sessions each 0.5 h following 1 and 5 mg/kg SCOP doses (1 and 5 mg/kg) with half tested with an ascending dose order and the remainder with a descending dose order. SCOP was mixed at concentrations of 0.1 and 0.5 mg/ml of 0.9% normal saline and administered i.p. at 10 ml/kg body weight. These doses were chosen based on the testing of sucrose-CFP expression in the three strains of mice (Iskhakov et al., 2018).

3.1.4. Intralipid-CFP acquisition procedure

BALB/c, C57BL/6 and SWR mice received 10 one-bottle training sessions (1 h/day) with the CS+/I solution presented on odd-numbered days, and the CS−/i solution presented on even-numbered days. Thirty min prior to each of the one-bottle training trials, Veh or SCOP injections (i.p.) were administered to subgroups of BALB/c (Veh (n = 8); SCOP 2.5 (n = 10); SCOP 5 (n = 10)), C57BL/6 (Veh (n = 10); SCOP 2.5 (n = 10); SCOP 5 (n = 10)) and SWR (Veh (n = 8); SCOP 2.5 (n = 10); SCOP 5 (n = 10)) mice. These two SCOP doses were identical to those tested for sucrose-CFP acquisition in these inbred mouse strains (Iskhakov et al., 2018). Following training, all mice were given six daily 2-bottle choice sessions (1 h/day) with the CS+ and CS− 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) to control for side position effects.

3.1.5. Statistics

In CFP expression, training intakes of the average of the five CS+/I and five CS−/i sessions were determined, and evaluated for strain and CS differences using a two-way analysis of variance (ANOVA). Expression of Intralipid-CFP in 2-bottle preference vehicle tests was evaluated in two-way ANOVAs among the three strains and CS+ vs. CS− intakes. Subsequent two-way ANOVAs evaluated drug effects within each strain (CS solution vs. Dose) for the Intralipid-CFP expression paradigm. Percent CS+ preference was calculated for each animal in the following manner: CS+ intake / Total Intake × 100. Separate ANOVAs evaluated percent CS+ intakes as a function of drug dose for each strain as well as comparing the three strains. In acquisition, training intakes of the average of the five CS+/I and five CS−/i sessions were determined, and were evaluated for strain and CS effects using a two-way ANOVA. A three-way ANOVA compared the CS intakes of the strains (Group × CS × Test). Separate two-way ANOVAs evaluated percent CS+ intakes within strains, 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. Strain-specific effects were evaluated for %CS+ intakes in Intralipid-CFP expression using a 2-way ANOVA examining strains and dose conditions. Strain-specific effects were evaluated for %CS+ intakes in Intralipid-CFP acquisition expression using a 3-way ANOVA examining strains, dose and test conditions. When main or interaction effects were found, Tukey comparisons ($p < 0.05$) were employed to detect significant effects.

3.2. Results

3.2.1. Intralipid-CFP expression training intakes

In the expression experiment, significant differences in one-bottle training intakes were observed among the three strains ($F(2,18) = 10.03$, $p = 0.001$) and between the CS+/I and CS−/i conditions ($F(1,9) = 176.53$, $p = 0.0001$), but not for the interaction between strains and conditions ($F(2,18) = 1.64$). CS+/I training intake was significantly greater than CS−/i training intake for BALB/c, C57BL/6 and SWR strains (Fig. 2A). CS+/I and CS−/i training intakes were significantly lower in BALB/c relative to C57BL/6 and SWR mice (Fig. 2A). In turn, Intralipid-CFP training intakes failed to differ between C57BL/6 and SWR mice. Therefore, because strain-specific differences in intakes occurred, consistent with previous studies (Iskhakov et al., 2018; Kraft et al., 2013, 2017), muscarinic cholinergic antagonist effects on intakes in the expression of Intralipid-CFP were evaluated separately for each strain.

3.2.2. SCOP and Intralipid-CFP expression in BALB/c mice

Significant differences in two-bottle choice intakes were observed between the CS+ and CS− conditions ($F(1,27) = 13.76$, $p = 0.001$), approached significance among SCOP doses ($F(2,27) = 3.05$, $p = 0.064$), but not for the interaction between doses and conditions ($F(2,27) = 1.57$). CS+ intake was significantly greater than CS− intake in BALB/c mice following vehicle, but not following the 1 and 5 mg/kg SCOP doses (Fig. 2B). The higher, but not lower SCOP dose significantly lowered CS+, but not CS− intake relative to vehicle treatment (Fig. 2B). Percent CS+ intake failed to differ for Intralipid-CFP expression ($F(2,18) = 1.75$) following vehicle (70%), and the 1 (67%) and 5 (58%) mg/kg SCOP doses (Fig. 2B). Differences in total intake approached significance ($F(2,18) = 3.50$, $p = 0.052$), but intakes following vehicle (1.1 g) and the 1 (0.8 g) and 5 (0.6 g) mg/kg SCOP doses failed to differ from each other.

3.2.3. SCOP and Intralipid-CFP expression in C57BL/6 mice

Significant differences in two-bottle choice intakes were observed among SCOP doses ($F(2,27) = 9.13$, $p = 0.0009$), between the CS+ and CS− conditions ($F(1,27) = 44.72$, $p = 0.0001$) and for the interaction between doses and conditions ($F(2,27) = 11.21$, $p = 0.0003$). CS+ intake was significantly greater than CS− intake following vehicle, but not either SCOP dose (Fig. 2C). Both SCOP doses significantly reduced CS+, but not CS− intakes (Fig. 2C). Significant differences in percent CS+ intake were observed ($F(2,18) = 4.46$, $p = 0.027$) with the lower (1 mg/kg: 56%), but not the higher (5 mg/kg: 60%) lower than vehicle (71%) treatment (Fig. 2C). Significant differences in total intake were observed ($F(2,18) = 11.61$, $p = 0.0006$) with intake following the 1 (1.2 g) and 5 (1.1 g) mg/kg SCOP doses significantly lower than vehicle (2.1 g) treatment.

3.2.4. SCOP and Intralipid-CFP expression in SWR mice

Significant differences in two-bottle choice intakes were observed among SCOP doses ($F(2,27) = 8.84$, $p = 0.001$), between the CS+ and CS− conditions ($F(1,27) = 65.43$, $p = 0.0001$) and for the interaction between doses and conditions ($F(2,27) = 4.72$, $p = 0.017$). CS+ intake was significantly greater than CS− intake in all drug treatments (Fig. 2D). SCOP significantly reduced CS+, but not CS− intake relative to vehicle (Fig. 2D). Further, percent CS+ intake failed to differ significantly ($F(2,18) = 1.61$) following vehicle (80%) and the 1 (74%) and 5 (68%) mg/kg SCOP doses (Fig. 2D). However, significant differences in total intake were observed ($F(2,18) = 10.36$, $p = 0.001$) with intake following the 1 (1.2 g) and 5 (0.9 g) mg/kg SCOP doses significantly lower than vehicle (1.8 g) treatment.

3.2.5. Strain differences in percent CS+ intake in Intralipid-CFP expression following SCOP

Significant differences in percent CS+ intake were observed among strains ($F(2,18) = 7.07$, $p = 0.005$) and among dose treatments ($F(2,18) = 7.76$, $p = 0.004$), but not for the interaction between strains and doses ($F(4,36) = 0.63$). However, percent CS+ intake failed to differ among strains when comparing among vehicle, SCOP 1 and SCOP 5 treatments.

3.2.6. Intralipid-CFP acquisition training and vehicle testing intakes

In the acquisition experiment, significant differences in one-bottle training intakes were observed among the three strains ($F(2,18) = 8.63$, $p = 0.002$), between the CS+/I and CS−/i conditions ($F(1,9) = 443.66$, $p = 0.0001$) and for the interaction between strains and conditions ($F(2,18) = 14.65$, $p = 0.0002$). CS+/I intake was

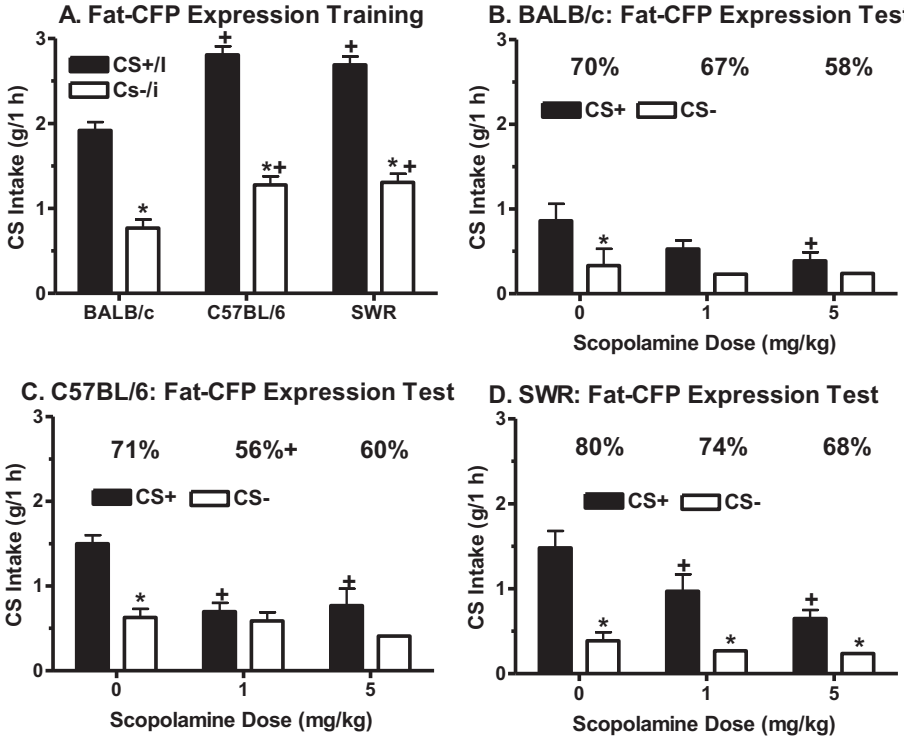

Fig. 2. Fat-CFP Expression Training and Testing: Intakes (mean, ± SEM, g/1 h) of two concentrations of Intralipid (5%, CS + /I; 0.5%, CS − /i) solutions during one-bottle training (Panel A). Significant differences between CS + /I and CS − /i training intakes within strains (*) and between CS + /I and CS − /I intakes between strains (+) are denoted. Intakes (mean, ± SEM, g/1 h) of CS + and CS − solutions in 2-bottle tests in the fat-CFP experiments in BALB/c (Panel B), C57BL/6 (Panel C) and SWR (Panel D) inbred mice receiving systemic injections of the muscarinic cholinergic antagonist, scopolamine 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 percentage CS + intake ((CS + intake/total intake) × 100) is denoted above each pair of values with significant differences relative to vehicle treatment (+).

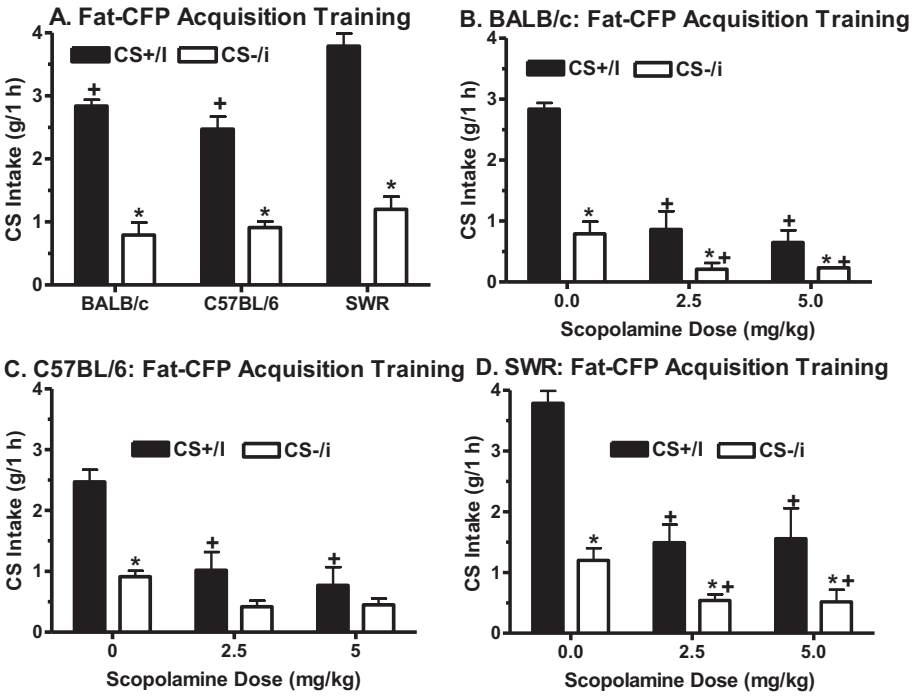

Fig. 3. Fat-CFP Acquisition Training: Training intakes (mean, ± SEM, g/1 h) of 5% Intralipid (CS + /I) and 0.5% Intralipid (CS − /i) solutions in BALB/c, C57BL/6 and SWR mice pretreated 30 min earlier with vehicle (Panel A). Significant differences between CS + /I and CS − /i training intakes are denoted within strains (*) as well as significant differences in CS + /I intake relative to SWR mice (+). Training intakes (mean, ± SEM, g/1 h) of 5% Intralipid (CS + /I) and 0.5% Intralipid (CS − /i) solutions in BALB/c (Panel B), C57BL/6 (Panel C) and SWR (Panel D) mice pretreated 30 min earlier with vehicle (0.0) or scopolamine at doses of 2.5 and 5 mg/kg. Significant differences between CS + /I and CS − /i training intakes are denoted within strains (*) as well as CS + /I and CS − /i intakes between vehicle and antagonist doses (+).

significantly higher than CS − /i intake in BALB/c, C57BL/6 and SWR mice (Fig. 3A). SWR mice consumed significantly more CS + /I, but not CS − /i intake than BALB/c and C57BL/6 mice (Fig. 3A). In 2-bottle tests following vehicle, significant differences were observed among the three strains ($F(2,18) = 17.40$, $p = 0.0001$), and between the CS + and CS − solutions ($F(1,9) = 187.86$, $p = 0.0001$), but not among tests ($F(2,18) = 2.04$). Significant interactions were observed between and strains and conditions ($F(2,18) = 22.97$, $p = 0.0001$), but not between strains and tests ($F(4,36) = 0.50$), tests and conditions ($F(2,18) = 1.28$) or among strains, tests and conditions ($F(4,36) = 1.79$). CS + intake was significantly higher than CS − intake across all three tests following vehicle in BALB/c (Fig. 4A), C57BL/6 (Fig. 5A) and SWR (Fig. 6A) mice. SWR mice consumed significantly more CS + solution across all three tests following vehicle treatment than BALB/c and C57BL/6 mice (Fig. 3A). In turn, C57BL/6 mice consumed significantly more CS − solution after the second test following vehicle treatment than BALB/c or SWR mice. Therefore, because strain-specific differences in intakes occurred in one-bottle training and in 2-bottle testing following vehicle, muscarinic cholinergic antagonist effects on intakes in the acquisition of Intralipid-CFP were evaluated separately for each strain.

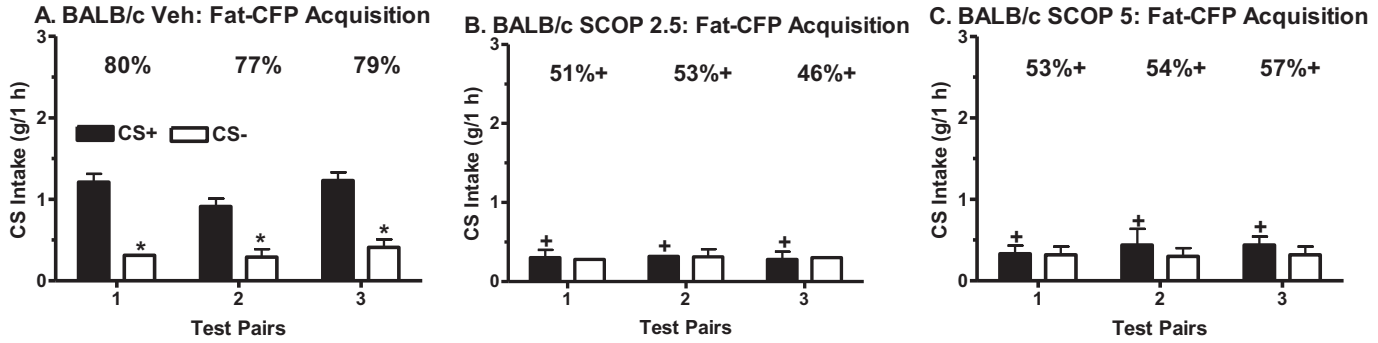

Fig. 4. Fat-CFP Acquisition Testing in BALB/c Mice: Intakes (mean, ± SEM, g/1 h) of CS+ and CS− solutions during 2-bottle Tests 1, 2 and 3 in BALB/c mice receiving vehicle (Veh: Panel A) or scopolamine (SCOP) at doses of 2.5 (Panel B) or 5 (Panel C) mg/kg during training. Significant differences are denoted between CS + and CS− intake (*) and for drug treatment relative to corresponding Veh (+) in this and the following two figures. The percentage CS+ intake ((CS+ intake / total intake) × 100) is denoted above each pair of values with significant differences relative to vehicle treatment (+) in this and the following two figures.

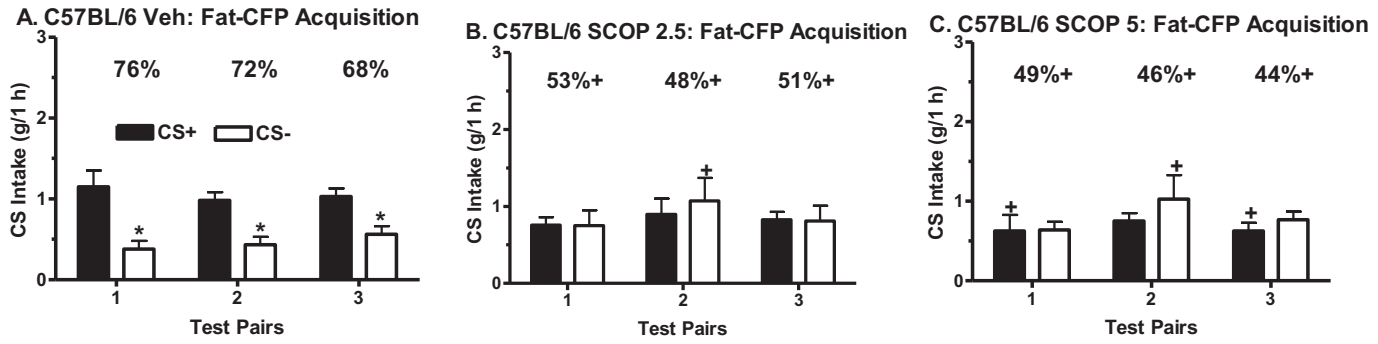

Fig. 5. Fat-CFP Acquisition Testing in C57BL/6 Mice: Intakes (mean, ± SEM, g/1 h) of CS+ and CS− solutions during 2-bottle Tests 1, 2 and 3 in C57/BL/6 mice receiving Veh (Panel A) or SCOP at doses of 2.5 (Panel B) or 5 (Panel C) mg/kg during training.

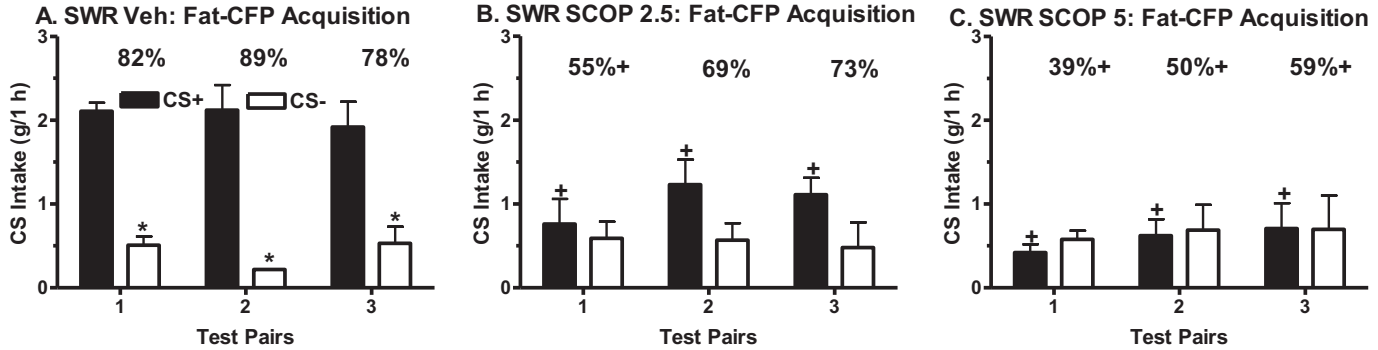

Fig. 6. Fat-CFP Acquisition Testing in SWR Mice: Intakes (mean, ± SEM, g/1 h) of CS+ and CS− solutions during 2-bottle Tests 1, 2 and 3 in SWR mice receiving Veh (Panel A) or SCOP at doses of 2.5 (Panel B) or 5 (Panel C) mg/kg during training.

3.2.7. SCOP and Intralipid-CFP acquisition in BALB/c mice

In acquisition training, BALB/c mice displayed significant differences in intake among SCOP doses ($F(2,18) = 75.04$, $p = 0.0001$), between CS+ and CS− conditions ($F(1,9) = 65.62$, $p = 0.0001$) and for the interaction between doses and conditions ($F(2,18) = 28.18$, $p = 0.0001$). CS+/I intake was significantly higher than CS−/i intake following vehicle and both SCOP doses (Fig. 3B). CS+/I and CS−/i intakes were significantly lower than corresponding vehicle values in mice receiving the 2.5 and 5 mg/kg SCOP doses (Fig. 3B). In two-bottle choice testing, BALB/c mice displayed significant differences in intake among doses ($F(2,18) = 45.26$, $p = 0.0001$), between CS+ and CS− conditions ($F(1,9) = 46.84$, $p = 0.0001$), but not among the three tests ($F(2,18) = 1.04$). Significant interactions were observed between doses and conditions ($F(2,18) = 38.32$, $p = 0.0001$) and among doses, tests and conditions ($F(4,36) = 2.65$, $p = 0.049$), but not between doses and tests ($(4,36) = 2.03$) and tests and conditions ($F(2,18) = 0.36$). CS+ intake was significantly higher than CS− intake across all three tests following vehicle treatment (Fig. 4A), but not following treatments with the 2.5 (Fig. 4B) or 5 (Fig. 4C) mg/kg SCOP doses. CS+, but not CS− intakes were significantly reduced in mice receiving the 2.5 (Fig. 4B) and 5 (Fig. 4C) mg/kg SCOP doses relative to vehicle-treated mice. BALB/c mice also displayed significant differences in %CS+ intake among doses ($F(2,18) = 12.87$, $p = 0.00031$), but not among tests ($F(2,18) = 0.17$) or for the interaction between doses and tests ($F(4,36) = 1.26$). The magnitude of Intralipid-CFP significantly declined across all three tests following treatment with the 2.5 (46–53%: Fig. 4B) and 5 (53–57%: Fig. 4C) mg/kg SCOP doses relative to vehicle (75–80%: Fig. 4A). BALB/c mice displayed significant differences in total intake among doses ($F(2,18) = 46.72$, $p = 0.0001$), but not among tests ($F(2,18) = 1.27$) or for the interaction between doses and tests F

(4,36) = 2.00). The 2.5 (0.5–0.6 g) and 5 (0.6–0.8 g) mg/kg SCOP doses significantly reduced total intake across the three tests relative to vehicle (1.2–1.6 g).

3.2.8. SCOP and Intralipid-CFP acquisition in C57BL/6 mice

In acquisition training, C57BL/6 mice displayed significant differences in intake among SCOP doses (F(2,18) = 12.30, p = 0.0004), between CS+ and CS− conditions (F(1,9) = 59.55, p = 0.0001) and for the interaction between doses and conditions (F(2,18) = 12.99, p = 0.0003). CS+/I intake was significantly higher than CS−/i intake following vehicle, but not either SCOP dose (Fig. 3C). CS+/I, but not CS−/i intake was significantly reduced following both SCOP doses relative to vehicle (Fig. 3C). In two-bottle choice testing, C57BL/6 mice displayed significant differences in intake among the three tests (F(2,18) = 3.69, p = 0.046), but not among doses (F(2,18) = 0.45) or and between CS+ and CS− conditions (F(1,9) = 2.74). Significant interactions were observed between doses and conditions (F(2,18) = 12.69, p = 0.0004), but not between doses and tests (4,36) = 2.30), between tests and conditions (F(2,18) = 2.00) or among doses, tests and conditions (F(4,36) = 0.39). CS+ intake was significantly higher than CS− intake across all three tests following treatment with vehicle (Fig. 5A), but not following treatment with the 2.5 (Fig. 5B) and 5 (Fig. 5C) mg/kg SCOP doses. CS+ intake was significantly lower in mice treated with the SCOP 5 mg/kg dose after the first and third tests relative to vehicle (Fig. 5C). CS− intake was significantly higher in mice treated with the SCOP 2.5 (Fig. 5B) and 5 (Fig. 5C) mg/kg doses after the second test relative to vehicle. Percent CS+ intake was significantly different among doses (F(2,18) = 33.18, p = 0.0001), but not among tests (F(2,18) = 1.59) or for the interaction between doses and tests (F(4,36) = 0.31). The magnitude of Intralipid-CFP was significantly lower across all three tests following treatment with the 2.5 (48–53%: Fig. 5B) and 5 (44–49%: Fig. 5C) mg/kg SCOP doses relative to vehicle (68–76%: Fig. 5A). Total intake failed to differ among doses (F(2,18) = 0.40), among tests (F(2,18) = 3.34) and for the interaction between doses and tests F(4,36) = 2.38).

3.2.9. SCOP and Intralipid-CFP acquisition in SWR mice

In acquisition training, SWR mice displayed significant differences in intake among SCOP doses (F(2,18) = 17.07, p = 0.0001), between CS+ and CS− conditions (F(1,9) = 57.36, p = 0.0001) and for the interaction between doses and conditions (F(2,18) = 29.60, p = 0.0001). CS+/I intake was significantly higher than CS−/i intake only following vehicle and both SCOP doses (Fig. 3D). CS+/I and CS−/i intakes were significantly lower than vehicle values following both SCOP doses (Fig. 3D). In two-bottle choice testing, SWR mice displayed significant differences in intake among doses (F(2,18) = 7.71, p = 0.004) and between CS+ and CS− conditions (F(1,9) = 27.72, p = 0.0005), but not or among the three tests (F(2,18) = 0.64). Significant interactions were observed between and doses and conditions (F(2,18) = 10.82, p = 0.0008), but not between doses and tests (4,36) = 1.16), between tests and conditions (F(2,18) = 1.67) or among doses, tests and conditions (F(4,36) = 1.22). CS+ intake was significantly higher than CS− intake across all three tests following treatment with vehicle (Fig. 6A), but not the 2.5 (Fig. 6B) or 5 (Fig. 6C) mg/kg SCOP doses. CS+, but not CS− intake was significantly lower across all three tests following both SCOP doses relative to vehicle (Fig. 6). SWR mice displayed significant differences in %CS+ intake among doses (F(2,18) = 6.72, p = 0.007), among tests (F(2,18) = 7.70, p = 0.004) and for the interaction between doses and tests (F(4,36) = 3.20, p = 0.024). The magnitude of Intralipid-CFP significantly declined relative to vehicle (78–89%: Fig. 6A) following the first test (55%) following the 2.5 mg/kg SCOP dose (Fig. 6B) and following all three tests following the 5 mg/kg SCOP dose (Fig. 6C). Finally, SWR mice displayed significant differences in total intake among doses (F(2,18) = 8.13, p = 0.003) but not among tests (F(2,18) = 0.62) or for the interaction between doses and tests F(4,36) = 1.19). Total intake of SWR mice significantly decreased during the first and third tests following the 2.5 mg/kg SCOP dose and following all three tests following the 5 mg/kg SCOP dose relative to vehicle.

3.2.10. Strain differences in percent CS+ intake in Intralipid-CFP acquisition following SCOP

Significant differences in percent CS+ intake were observed among strains (F(2,18) = 5.23, p = 0.016) and doses (F(2,18) = 28.83, p = 0.0001), but not among tests (F(2,18) = 0.75). Significant interactions were observed between strains and tests (F(4,36) = 4.32, p = 0.006) and doses and tests (F(4,36) = 3.70, p = 0.013), but not between strains and doses (F(4,36) = 1.33) or among strains, doses and tests (F(8,72) = 1.10). Percent CS+ intakes in mice receiving vehicle during training failed to differ among strains (F(2,18) = 16.43, p = 0.0001). The percent CS+ intakes of SWR mice receiving the 2.5 (Fig. 6B: 69% and 73%), but not 5 mg/kg SCOP dose during training were significantly higher for the second and third tests than their BALB/c (Fig. 4B: 53% and 46%) and C57BL/6 (Fig. 5B: 48% and 51%) counterparts.

4. Discussion

The present study examined whether muscarinic cholinergic receptor antagonism with SCOP differentially altered fat (Intralipid) intake as well as the acquisition and expression of fat (Intralipid)-CFP in male BALB/c, SWR and C57BL/6 mice. Before proceeding, one major caveat of the study is the failure to include female animals. As indicated in recent reviews of murine strain differences in food intake (Bodnar et al., 2013) and CFP (Bodnar, 2018), virtually all of the studies in the literature have used male mice. One reason for this is that, in addition to comparing male and female animals for sex differences in intake, female animals must be tested across different stages of the reproductive cycle as animals during the estrus phase and ovariectomized animals respectively display decreases and increases in different forms of food intake (Becker et al., 2005; Eckel, 2011; Eckel and Moore, 2004; Eckel et al., 2005; Santollo and Eckel, 2008). Ackroff and Sclafani (1991) found that although both males and female rats developed a preference for a glucose-paired flavored food over a fructose-paired flavor food, this preference emerged more rapidly in males relative to females. Ackroff and Sclafani (2004) also found that although both sexes preferred a sweet saccharin flavor paired with intragastric fructose, a non-sweet flavor paired with intragastric fructose elicited avoidance in male rats and indifference in female rats. Moreover, in choice tests between sucrose and Polycose, female rats preferred the former, and male rats preferred the latter in short-term choice tests (Sclafani et al., 1987). Our laboratory (LaMagna et al., 2019) found that although the magnitudes of sucrose-CFP were comparable in two-bottle choice tests in young adult BALB/c and C57BL/6 male and female mice exposed to water during adolescence, young adult BALB/c male and female mice displayed significantly greater sucrose-CFP preferences relative to their C57BL/6 male and female counterparts when both strains were exposed to sucrose or saccharin during adolescence. No systematic sex differences were observed in these responses. It is important in future studies to evaluate whether sex and estrous phase differences are present in the pharmacological substrates of fat intake and fat-CFP in these strains.

4.1. SCOP and fat intake in BALB/c, C57BL/6 and SWR inbred mice

The first experiment found that all doses of SCOP significantly decreased fat (Intralipid) intake to a similar degree in all three strains (Table 1). The scopolamine dose range employed in the present study was identical to that previously evaluated in outbred rats (Rotella et al., 2015) and these three inbred mouse strains (Bourie et al., 2017). Therefore, it is not known whether the suppression induced by the

Table 1
Summary of strain differences in the inhibition (ID_{40}) by cholinergic, opioid and dopamine D1 receptor antagonists on fat (Intralipid) intake.

Antagonist	BALB/c	C57BL/6	SWR
Scopolamine	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Naltrexone	14 mg/kg	4.4 mg/kg	4 mg/kg
SCH23390	1928 nmol/kg	598 nmol/kg	203 nmol/kg

highest (10 mg/kg) scopolamine dose was maximal. However, the similar pattern of muscarinic cholinergic antagonist effects upon Intralipid-induced intake in the three strains is in marked contrast to previously-observed strain-specific effects observed for SCOP-induced decreases in nutritive (sucrose) and non-nutritive (saccharin) intake (Bourie et al., 2017). SCOP effectively reduced sucrose intake in BALB/c and C57BL/6 mice more potently than in SWR mice (Table 1). Correspondingly, BALB/c and C57BL/6 mice produced more potent and significant reductions in saccharin intake following SCOP than SWR mice (Table 1; Bourie et al., 2017; Kraft et al., 2015). Thus, SCOP-induced reductions in fat intake are more pronounced and not subject to strain-specific effects. Previous studies demonstrated a differential presence of murine strain differences in the inhibition of fat (intake following systemic administration of opioid (naltrexone) and dopamine D1 (SCH23390) receptor antagonists in BALB/c, C57BL/6 and SWR inbred mouse strains (Dym et al., 2010). Thus, naltrexone significantly reduced Intralipid intake in SWR and C57BL/6, but not in BALB/c mice (Table 1; Dym et al., 2010). Similarly, SCH23390 significantly and more potently reduced Intralipid intake in SWR relative to C57BL/6 mice, but not in BALB/c mice (Table 1; Dym et al., 2010). Hence, murine strain differences appear to affect the neurochemical substrates of fat (Intralipid) intake with greater sensitivity to muscarinic cholinergic receptor antagonism in BALB/c and C57BL/6 mice relative to SWR mice, to opioid receptor antagonism in SWR and C57BL/6 mice relative to BALB/c mice, and to dopamine D1 receptor antagonism in SWR mice relative to C57BL/6 mice and BALB/c mice (Table 1).

4.2. SCOP and Fat-CFP expression in BALB/c, C57BL/6 and SWR inbred mice

The second experiment of the present study examined whether muscarinic cholinergic receptor antagonism with SCOP differentially altered the expression (maintenance) and acquisition (learning) of fat (Intralipid)-CFP in BALB/c, SWR and C57BL/6 mice. Robust fat-CFP following vehicle treatment in the expression paradigm was evident in BALB/c (70%) and SWR (80%) mice consistent with previous findings in these strains (Kraft et al., 2013, 2017). C57BL/6 (71%) mice also displayed a robust and consistent fat-CFP following vehicle treatment. SCOP produced small, but significant effects on the expression of fat-CFP in BALB/c and C57BL/6 mice. Whereas C57BL/6 mice failed to display differences between CS+ and CS− intake following SCOP and the magnitude of fat-CFP was reduced following the lower SCOP dose, the magnitude of fat-CFP following the higher SCOP dose failed to differ significantly from vehicle values. Whereas BALB/c mice failed to display differences between CS+ and CS− intake following SCOP, the magnitude of fat-CFP following both SCOP doses failed to differ significantly from vehicle values. In contrast, the expression of fat-CFP was unaffected by SCOP in SWR mice as demonstrated by significantly greater CS+ relative to CS− intake under all conditions as well as the observation that the magnitude of fat-CFP following both SCOP doses failed to differ significantly from vehicle values. Alterations in the expression of fat-CFP also differed following dopamine D1, opioid and NMDA receptor antagonism in these three strains (Kraft et al., 2013, 2017). Whereas the expression of fat-CFP in BALB/c mice was eliminated by dopamine D1, opioid and NMDA receptor antagonism, SWR mice displayed strong significant reductions in this response following dopamine D1 and NMDA, but not opioid receptor antagonism (Table 2; Kraft et al., 2013, 2017). Our laboratory (Bodnar et al., unpublished study) recently found that small, but significant reductions in the expression of fat-CFP were observed following dopamine D1, opioid and NMDA receptor antagonism in C57BL/6 mice (Table 2). Collectively, these data indicate that single administration of dopamine D1, opioid or NMDA, but not muscarinic cholinergic receptor antagonists eliminate the expression of fat-CFP in BALB/c mice. Expression of fat-CFP in SWR mice is eliminated by dopamine D1 receptor antagonism, significantly reduced by NMDA receptor antagonism, but unaffected by muscarinic cholinergic or opioid receptor antagonism. In contrast, C57BL/6 mice typically display small, significant reductions in the expression of fat-CFP following administration of muscarinic cholinergic, dopamine D1, opioid or NMDA receptor antagonism, demonstrating a strong strain-specific sensitivity and insensitivity to pharmacological antagonism of the maintenance of an already-learned fat preference.

4.3. SCOP and fat-CFP acquisition in BALB/c, C57BL/6 and SWR inbred mice

The present study demonstrated that muscarinic cholinergic receptor antagonism produced far greater effects upon the acquisition of fat-CFP in the three strains as compared to the expression of fat-CFP. Whereas SCOP either minimally (BALB/c, C57BL/6) or failed to affect (SWR) fat-CFP, expression, muscarinic cholinergic receptor antagonism eliminated the ability to learn fat-CFP in the three strains. The ability of SCOP to potently inhibit fat intake in the three freely-feeding mouse strains in the first experiment was again observed in the three food-restricted mouse strains receiving SCOP during one-bottle training. CS+/I intake was significantly reduced by both SCOP doses in all strains, and CS−/I intake was significantly reduced by both SCOP doses in BALB/c and SWR mice. Table 2 summarizes other pharmacological interventions upon the acquisition of fat-CFP in the three strains. Fat-CFP acquisition was eliminated in SWR mice by SCH23390, but not naltrexone, and was only mildly affected by both antagonists in BALB mice (Kraft et al., 2013). MK-801 eliminated acquisition of fat-CFP in BALB/c mice, and actually changed the preference to an avoidance response in SWR mice (Kraft et al., 2017). Finally, preliminary data from our laboratory indicated that C57BL/6 mice displayed at best

Table 2
Summary of murine strain-specific effects on the magnitude of pharmacological antagonist-induced effects upon acquisition and expression of fat-CFP.

CFP	Strain	Scopolamine	SCH23390	Naltrexone	MK-801
Condition					
Acquisition	BALB/c	50% or below	No effect[1]	50% or below[1]	50% or below[2]
	C57BL/6	50% or below	Over 60%[3]	No effect[3]	Over 60%[3]
	SWR	50% or below	50% or below[1]	No effect[1]	50% or below[2]
Expression	BALB/c	Over 60%	50% or below[1]	50% or below[1]	50% or below[2]
	C57BL/6	Over 60%	Over 60%[3]	Over 60%[3]	Over 60%[3]
	SWR	No effect	50% or below[1]	No effect[1]	50–60%[2]

Note 1: Significant fat-CFP preferences following vehicle treatment were between 67 and 80% across paradigms and strains. Note 2: [1]Kraft et al., 2013; [2]Kraft et al., 2017; [3]unpublished results.

minimal reductions in the acquisition of fat-CFP following SCH23390 and MK-801, but not naltrexone. The sites of action at which these differential strain-specific pharmacological actions take place and the underlying mechanisms mediating these differences among strains need to be elucidated in future studies.

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