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Effect of dopamine D1 and D2 receptor antagonism in the lateral hypothalamus on the expression and acquisition of fructose-conditioned flavor preference in rats

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Abstract

The attraction to sugar-rich foods is influenced by conditioned flavor preferences (CFP) produced by the sweet taste of sugar (flavor-flavor learning) and the sugar's post-oral actions (flavor-nutrient) learning. Brain dopamine (DA) circuits are involved in both types of flavor learning, but to different degrees. This study investigated the role of DA receptors in the lateral hypothalamus (LH) on the flavor-flavor learning produced the sweet taste of fructose. In an acquisition study, food-restricted rats received bilateral LH injections of a DA D1 receptor antagonist (SCH23390), a D2 antagonist (RAC, raclopride) or vehicle prior to 1-bottle training sessions with a flavored 8% fructose + 0.2% saccharin solution (CS+/F) and a less-preferred flavored 0.2% saccharin solution (CS-). Drug-free 2-bottle tests were then conducted with the CS+ and CS- flavors presented in saccharin. The fructose-CFP did not differ among groups given vehicle (76%), 12 nmol SCH (78%), 24 nmol (82%) or 24 nmol RAC (90%) during training. In an expression study with rats trained drug-free, LH injections of 12 or 24 nmol SCH or 12-48 nmol RAC prior to 2-bottle tests did not alter CS+ preferences (77-90%) relative to vehicle injection (86%). Only a 48 nmol SCH dose suppressed the CS+ preference (61%). The minimal effect of LH DA receptor antagonism upon fructose flavor-flavor conditioning differs with the ability of LH SCH injections to block the acquisition of glucose flavor-nutrient learning.

Keywords

Flavor-flavor learning; sweet taste; saccharin; SCH23390; Raclopride

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1. Introduction

Learning plays an important role in the development of food and fluid preferences. Preferences for novel flavors can be learned based on associations between that flavor and an already preferred flavor (e.g., sweet taste of sugar) and/or the post-oral reinforcing properties of a nutrient (e.g., glucose). These processes are respectively referred to as flavor-flavor and flavor-nutrient conditioning (Sclafani, 1995). We have studied flavor-flavor conditioning, the subject of the present study, by training food-restricted rats to drink a flavor (the CS+, e.g., grape) mixed into a preferred sugar solution and an alternative flavor (the CS-, e.g., cherry) mixed into a less preferred saccharin solution during daily one-bottle sessions, and then assessing preferences in two-bottle choice tests with the CS+ and CS- flavors presented in a saccharin solution. In initial studies, flavor-flavor conditioning was produced using sucrose in a sham-feeding procedure to minimize the post-oral nutrient reinforcement (Yu et al., 2000a, 2000b). Subsequent studies used fructose in a real-feeding procedure (Baker et al., 2003) based on the finding that fructose does not support post-oral flavor conditioning in rats with short training sessions (Sclafani and Ackroff, 1994; Sclafani et al., 1993, 1999). Parallel flavor-nutrient conditioning studies were conducted with a CS+ flavor paired with an intragastric (IG) infusion of sucrose or glucose and a CS- flavor paired with an IG water infusion (see reviews: Sclafani et al., 2011; Touzani et al., 2010b).

Brain dopamine (DA) systems are differentially implicated in the acquisition and expression of flavor-flavor and flavor-nutrient preferences. In particular, systemic administration of DA D1-like (SCH23390, SCH) or D2-like (raclopride, RAC) receptor antagonists reduced the acquisition and expression of flavor-flavor conditioning by sucrose and fructose (Baker et al., 2003; Yu et al., 2000a, 2000b). In contrast, only systemic SCH blocked IG sucrose-conditioned flavor-nutrient preferences and SCH and RAC had minimal or no effects on the expression of the learned preferences (Azzara et al., 2001). Subsequent studies revealed selective effects on flavor-flavor and flavor nutrient conditioning of drug microinfusions into the nucleus accumbens (NAc), amygdala (AMY) and medial prefrontal cortex (mPFC) which receive DA projections from the ventral tegmental area (VTA) (e.g., Swanson, 1982). Whereas SCH or RAC administered into the NAc significantly reduced expression of fructose-CFP, NAc administration of SCH or RAC during training failed to prevent initial acquisition of a fructose-CFP, but elicited more rapid extinction (Bernal et al., 2008; Malkusz et al., 2012). Correspondingly, AMY administration of SCH and RAC significantly reduced expression of fructose-CFP, whereas AMY administration of RAC, but not SCH blocked fructose-CFP acquisition (Bernal et al., 2009b; Malkusz et al., 2012). Administration of SCH or RAC into the mPFC blocked acquisition, but not expression of fructose-CFP (Malkusz et al., 2012). In flavor-nutrient conditioning, the acquisition of IG glucose-CFP was blocked by SCH (12 nmol) administration during training into the NAc (Touzani et al., 2008), AMY (Touzani et al., 2009a) and mPFC (Touzani et al., 2010a).

Whereas the NAc, AMY and mPFC receive DA projections from the ventral tegmental area (VTA) (e.g., Swanson, 1982), DA D1 and D2 receptors in the LH (e.g., Bubser et al., 2005; Mansour et al., 1990, 1992; Wamsley et al., 1989, 1992) receive DA innervation from the A13 DA-containing cells in the neighboring zona incerta (e.g., Eaton et al., 1994; Wagner et al., 1995). The LH plays a crucial role in the modulation of feeding and food-related learning and aversions (see reviews: Bures et al., 1998; Scalera, 2002). Classic studies demonstrated that LH neurons of monkeys trained to lick sweet solutions are activated by the sight of food (Rolls et al., 1976) that are modulated by learning (Mora et al., 1976) and hunger (Burton et al., 1976), and actually precede the animal's response to the sweet stimulus (Rolls et al., 1979). Specific roles for the LH itself and LH DA signaling have been demonstrated for flavor-nutrient CFP learning such that both LH lesions and DA D1-like

receptor antagonism within this area attenuated flavor preference learning induced by the post-oral reinforcing actions of nutrients (Touzani & Sclafani, 2001; 2002; Touzani et al., 2009b). These findings make the LH another important brain site to analyze in establishing the location at which DA receptor antagonists affect the expression and acquisition of fructose-CFP. To this end, SCH and RAC were administered into LH sites either during acquisition training or expression testing sessions.

2. Results

2.1. Histological Verification

Figure 1 is a schematic representation (Paxinos and Watson, 2009) of the bilateral cannula placements ($n=106$) of all 53 animals in the acquisition and expression studies. All cannulae were localized within the mid-caudal LH at the levels of the hypothalamic ventromedial and dorsomedial nuclei and the levels of the median eminence and arcuate nuclei. The distributions of cannulae of animals in the acquisition studies administered vehicle ($n=18$), SCH12 ($n=20$), SCH24 ($n=22$) or RAC24 ($n=22$) and in the expression studies administered SCH ($n=12$) or RAC ($n=12$) displayed considerable overlap with respect to one another within the mid-caudal LH. Moreover, these cannula placements displayed considerable overlap with those in the study (Touzani et al., 2009b) evaluating D1 antagonist effects upon IG glucose flavor-nutrient CFP.

2.2. LH D1 and D2 Antagonists and Acquisition of Fructose-CFP

Training intakes were limited to 16 ml/session to minimize the difference between CS+/F and CS− intakes as described previously (see reviews: Sclafani et al., 2011; Touzani et al., 2010b). In the 1-bottle training sessions, overall, CS+/F intake (13.3 g/1 h) exceeded CS− intake (10.0 g/1 h, $F(1,10)= 47.94$, $p<0.0001$). Significant differences in training intakes failed to be observed among groups ($F(3,30)= 0.71$, ns) or for the interactions between groups and CS intakes ($F(3,30)= 1.81$, ns). Small, but significant differences were observed for CS+/F intake over CS− intake in the Veh (12.4 (± 0.6) vs. 9.8 (± 0.9) g/1 h) and RAC24 (14.9 (± 0.3) vs. 9.8 (± 0.8) g/1 h) groups, but not in the SCH12 (13.0 (± 1.2) vs. 10.1 (± 1.4) g/1 h) or SCH24 (13.0 (± 1.0) vs. 10.7 (± 1.7) g/1 h) groups.

In the 2-bottle preference tests, an *omnibus* ANOVA revealed significant differences in CS intakes ($F(1,10)= 239.65$, $p<0.0001$) and groups ($F(3,30)= 5.65$, $p<0.003$), but not among the three tests ($F(2,20)= 2.79$, ns). Significant interactions were observed between groups and tests ($F(6,60)= 6.03$, $p<0.034$) and CS intakes and tests ($F(2,20)= 4.99$, $p<0.018$), but not between groups and conditions ($F(3,30)= 1.54$, ns) or among groups, conditions and tests ($F(6,60)= 1.45$, ns). Overall, CS+ intakes exceeded CS− intakes, and overall CS intake was higher in the SCH12 training group (23.5 g) relative to the Veh (15.6 g), SCH24 (14.8 g) or RAC24 (15.4 g) training groups. This effect was largely attributed to the significantly higher CS+ intake in the SCH12 group in the first test relative to the Veh and other groups (Figure 2B). However, further within-group comparisons revealed that CS+ intake was significantly greater than CS− intake across all three tests in the four groups (Figure 2). Moreover, analysis of the percent CS+ data failed to reveal significant differences among groups ($F(3,30)= 2.66$, ns), across tests ($F(2,20)= 2.03$, ns), or for the interaction between groups and tests ($F(6,60)= 0.35$, ns) (Figure 2). Thus, the magnitude of the preferences were similar across tests for the Veh (74-79% (± 4.7 -6.9)), SCH 12 (75-81% (± 6.0 -7.7)), SCH24 (80-84% (± 4.4 -5.8)) and RAC24 (86-93% (± 0.9 -4.3)) groups. These data indicate that neither LH DA D1 nor D2 receptor antagonism altered the acquisition of the fructose-CFP.

2.3. LH D1 and D2 Antagonism and Expression of Fructose-CFP

During 1-bottle training, the mean intake of the CS+/F solution exceeded that of the CS− solution (11.9 (± 0.4) vs. 6.5 (± 0.8) g/30 min, $t(11) = 9.13$, $p < 0.0001$). In the 2-bottle preference tests conducted with the D1 group, overall, CS+ intakes exceeded CS− intakes ($F(1,20) = 62.18$, $p < 0.0001$) and intakes did not vary significantly as a function of drug dose ($F(3,20) = 0.86$, ns). CS+ intake was higher ($p < 0.05$) than CS− intake following the 0, 12 and 24 nmol SCH doses (Figure 3A). Although the interaction between CS and drug doses was not significant ($F(3,20) = 1.73$, ns), a *post hoc* analysis revealed that CS+ and CS− intakes did not differ at the 48 nmol SCH dose (Figure 3A). This is consistent with the finding that the percent CS+ intake at the 48 nmol dose (61% (± 7)) was significantly less ($F(3,15) = 5.60$, $p < 0.009$) than that of the 0 (86% (± 5)), 12 (81% (± 8)) and 24 (90% (± 4)) nmol SCH doses which did not differ (Figure 3). Total intake did not differ across the 0 (16.2 (± 1.6) g), 12 (18.1 (± 1.8) g), 24 (14.3 (± 1.3) g), and 48 (17.1 (± 2.3) g) nmol SCH doses.

In the 2-bottle preference tests conducted with the D2 rats, overall, CS+ intakes exceeded CS− intakes ($F(1,20) = 81.79$, $p < 0.0001$), but intakes failed to vary significantly across doses ($F(3,20) = 1.11$, ns) or for the interaction between CS conditions and doses ($F(3,20) = 0.47$, ns). CS+ intake was significantly higher than CS− intake following vehicle and all RAC doses (Figure 3B). Percent CS+ intakes at the 0 (86% (± 4)), 12 (77% (± 5)), 24 (78% (± 5)) and 48 (82% (± 6)) nmol RAC doses did not differ from each other (Figure 3B). Increases in total intake approached significance ($F(3,15) = 3.06$, $p = 0.06$) with systematic increases in total intake following the 12 (16.2 (± 2.4) g), 24 (19.3 (± 4.1) g) and 48 (19.6 (± 3.2) g) nmol doses of RAC relative to vehicle (13.1 (± 1.1) g).

3. Discussion

The purpose of the present study was to examine whether DA receptor signaling in the LH plays a role in the expression and acquisition of fructose-CFP which are discussed in the following sections.

3.1. LH D1 and D2 Antagonism and Acquisition of Fructose-CFP

If D1-like receptor antagonism in the LH acts similarly on the acquisition of all forms of sugar-CFP, it would be expected that SCH in the mid-caudal LH would block the acquisition of fructose-CFP, just as the 12 nmol SCH dose blocked the acquisition of CFP elicited by IG glucose (Touzani et al., 2009b). However, bilateral SCH administration of a 12 or 24 nmol dose into the mid-caudal LH failed to alter the acquisition of fructose-CFP, indicating an important difference between flavor-flavor-mediated and flavor-nutrient-mediated CFP learning. A 12 nmol dose of SCH administered into the NAcS or AMY also failed to disrupt the acquisition of a fructose-CFP (Bernal et al., 2008, 2009b). However, administration of a 24 nmol dose of SCH or RAC into the mPFC and 24 nmol RAC but not SCH in the AMY blocked the acquisition of fructose-CFP (Malkusz et al., 2012). In contrast, 24 nmol SCH or RAC infused in the NAcS had no effect on the learning of a fructose-CFP (Bernal et al., 2008). Thus, there are site-specific effects of DA receptor modulation on the acquisition of fructose-CFP. One explanation of such differences might be that LH DA transmission is involved in processing the acquisition of postoral signals from sugars but not from oral perception of these nutrients.

3.2. LH D1 and D2 Antagonism and Expression of Fructose-CFP

The only significant finding of the present study was that bilateral administration of a high 48 nmol dose of SCH, but not RAC into the mid-caudal level of the LH reduced the expression of fructose-CFP. The 48 nmol SCH dose significantly reduced the expression of fructose-CFP from 86% to 61%, which is an effect similar to that produced by SCH

treatment in the NacS (Bernal et al., 2008) and AMY (Bernal et al., 2009b) whereas SCH infused in the mPFC had no effect on the expression of a fructose-CFP (Malkusz et al., 2012). An earlier preliminary study (Bernal et al., 2009a) found that expression of fructose-CFP failed to be affected by the identical dose range of SCH or RAC administered into more rostral LH sites at the level of the paraventricular nucleus. Touzani and co-workers (2009b) found that SCH administered into the mid-caudal LH did not alter the expression of a flavor-nutrient IG glucose-CFP but only a 12 nmol SCH dose was investigated. Perhaps a higher 48 nmol SCH dose would block the expression of IG glucose-CFP.

It is possible that the 48 nmol SCH dose blocked the expression of the CS+ preference because it impaired the ability of the animals to discriminate between the CS+ and CS- flavors. This seems unlikely, however, because ibotenic acid lesions of the LH do not prevent rats from learning to prefer a CS+ flavor that is paired with IG infusions of nutrients (Touzani & Sclafani, 2001; 2002). Thus, the attenuated CS+ preference produced by the 48 nmol SCH dose appears to be due to a deficit in the maintenance of the classically-conditioned responses. The limited action of this high LH SCH dose in reducing fructose-CFP expression without any apparent dose-dependency at the lower 12 and 24 nmol doses raises the concern that this effect might not be mediated through DA D1 receptors. It should be noted that SCH23390 has very high selectivity for DA D1 and D5 receptors (Billard et al., 1984; Hyttel, 1983) and minimal binding actions at DA D2, α -1-adrenergic, muscarinic or histaminergic receptors (Hyttel et al., 1989). SCH23390 is more selective and specific at DA D1 receptor sites than other DA D1 compounds (SKF-83,959 or SCH39166: Zhang et al., 2009). Although SCH23390 displays binding affinities for 5-HT1C and 5-HT2A receptors, its actions at these sites are about 10-fold lower than that at the DA D1 receptor (Bourne, 2001; Hyttel, 1983; Taylor et al., 1991). The roles of 5-HT, and specifically of 5-HT1C and 5-HT2A receptor subtypes in mediating fructose-CFP are unknown, and should be investigated further. Thus, DA antagonist effects upon the expression of sugar-mediated CFP systematically vary as functions of the DA subtype (D1 vs D2), the CFP mechanism (flavor-flavor fructose vs. flavor-nutrient IG glucose) and the sites of action.

3.3. Possible LH Mechanisms of Action

These differences in differential DA receptor subtype involvement may also be due to the source of DA input. Whereas the source of DA input into the NAcS and AMY is largely derived from the VTA (e.g., Asan, 1997, 1998; Eliava et al., 2003; Lammel et al., 2008; Swanson, 1982), DA innervation of the LH is derived largely from A13 DA neurons in the neighboring zona incerta (Eaton et al., 1994; Wagner et al., 1995). Indeed, this A13 DA projection may explain the ability of DA D1 antagonism to reduce the expression of fructose-CFP following mid-caudal, but not rostral LH infusions as the densest LH projections are found at the level of the dorso-medial hypothalamic nucleus. How DA transmission in the mid-caudal LH modulates the expression of flavor-flavor preference learning is still unknown, but one candidate that deserves interest is LH orexin-containing neurons. Orexin-containing neurons can act on orexin receptors in VTA neurons (Korotkova et al., 2003). This orexin-mediated VTA system is involved in behavioral effects associated with reward-paired stimuli (Aston-Jones et al., 2009; Harris et al., 2005), but also innervates VTA DA cells that project to corticolimbic areas involved in reward and reward-related learning (Fadel & Deutch, 2002; Balcita-Pedicino & Sesack, 2007). It should be noted that LH orexin cells are regulated by DA (Korotkova et al., 2003; Bubser et al., 2005). Thus, it is possible that DA release in the LH during expression of flavor-flavor fructose-CFP and acquisition of flavor-nutrient IG glucose CFP learning (Touzani et al., 2009b) modulates the LH-VTA orexin projection, and thereby potentiates the activation of the mesolimbic DA system by nutrients and nutrient-associated flavor cues. Finally, the ability of DA D1 but not

D2-antagonists in the mid-caudal LH to significantly reduce the expression of fructose-CFP is also consistent with the specificity of DA receptor subtype effects upon taste avoidance learning (Caulliez et al., 1996; Fenu et al., 2001), and the greater densities of D1 receptors in the LH (Mansour et al., 1992; Wamsley et al., 1992). The differential DA D1 and D2 antagonist effects are also consistent with the ability of DA D1 antagonists to block increases in miniature excitatory post-synaptic current (mEPSC) frequency of LH orexin neurons elicited by low DA doses, and the ability of DA D2 antagonists to block reductions in mEPSC frequency of LH orexin neurons elicited by high DA doses (Alberto et al., 2006).

3.4. Summary and Possible Future Directions

CFP is an important factor driving sugar intake in animals, and its acquisition and expression are differentially mediated by orosensory (flavor-flavor) and post-ingestive (flavor-nutrient) mechanisms. Whereas DA D1 and D2 receptor signaling mediates the flavor-flavor component of sugar-CFP, only DA D1 receptors appear to be involved in the flavor-nutrient component of sugar-CFP (see reviews: Sclafani et al., 2011; Touzani et al., 2010b). This differential pattern of effects persists in analyzing critical structures mediating DA function including the NacS, AMY and mPFC with DA D1 and D2 receptor signaling involved in flavor-flavor-mediated fructose-CFP and DA D1 receptor signaling involved in flavor-nutrient-mediated IG glucose-CFP. Given the critical role of the LH in the modulation of feeding and food-related learning (see reviews: Bures et al., 1998; Scalera, 2002), this makes the LH a potentially compelling site of action that could mediate DA effects upon the flavor-flavor and flavor-nutrient components of CFP. Supporting this contention, both LH lesions and LH DA D1 receptor antagonism reduced flavor-nutrient-CFP induced by IG glucose (Touzani & Sclafani, 2001; 2002; Touzani et al., 2009b). However, the present results reveal a very limited role for LH DA receptors in flavor-flavor preference conditioning produced by fructose. It is conceivable that the LH is involved in this orosensory process, but blockade of multiple neurochemical mechanisms may be necessary. This has been demonstrated recently by the ability of co-administered doses of NMDA and DA D1 antagonists into the AMY to eliminate the acquisition of flavor-nutrient-mediated IG glucose-CFP (Touzani et al., 2013). Another possibility is that the administration of DA antagonists into multiple sites (e.g., LH and NacS or LH and AMY) might lead to elimination of flavor-flavor-mediated fructose-CFP.

4. Experimental Procedure

4.1. Subjects, Surgery and Histological Verification

Male Sprague-Dawley rats (260-300 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages and maintained on a 12:12 h light/dark cycle with chow (5001, PMI Nutrition International, Brentwood, MO) and water available ad libitum, except as noted below. The stereotaxic coordinates were aimed at the level of the mid-caudal LH, and were based on placements producing reductions in the acquisition of flavor-nutrient IG glucose-CFP following DA D1 antagonism (Touzani et al., 2009b). Each rat was pretreated with chlorpromazine (3 mg/kg, i.p.) and anesthetized with Ketamine HCl (120 mg/kg, i.m.). Stainless steel guide cannulae (26-gauge, Plastics One, Inc., Roanoke, VA) were aimed stereotaxically (Kopf Instruments) at bilateral placements in the LH using the following coordinates: incisor bar (0 mm), 3.0-3.3 mm posterior to the bregma suture, 3.3 mm lateral to and angled 10° towards the sagittal suture, and 8.8 mm from the top of the skull. The cannulae were secured to the skull by four anchor screws with dental acrylic. The animals were allowed at least two weeks to recover from stereotaxic surgery before behavioral testing began. At the end of the experiment, all rats were overdosed with an anesthetic (Euthasol) and were injected transcardially with potassium chloride (15 mg/ml, 0.9% saline). Transcardiac perfusions were performed with 0.9% normal saline followed by

10% buffered formalin. Coronal 40- μ m sections, stained with Cresyl violet, were examined by light microscopy by an observer unfamiliar with the behavioral data; only animals with confirmed cannula placements in each of the expression and acquisition paradigms in both experiments were included in the data analysis. The experimental protocols in all experiments 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.

4.2. Test Solutions

The training solutions consisted of 8% fructose (Sigma Chemical Co., St. Louis, MO) and 0.2% sodium saccharin (Sigma Chemical Co.) mixture or a 0.2% sodium saccharin solution, each flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY). Half of the rats in each group had the cherry flavor added to the fructose +saccharin solution and the grape flavor added to the saccharin only solution; the flavors were reversed for the remaining rats. In the two-bottle preference tests, the cherry and grape flavors were each presented in a 0.2% saccharin solution. The fructose+saccharin-paired flavor is referred to as the CS+, and the saccharin-paired flavor as the CS– because 8% fructose is preferred to 0.2% saccharin (Sclafani and Ackroff, 1994). CS+/F refers to the flavored fructose+saccharin solution used in training, and CS+ refers to the same flavor presented in saccharin only during choice testing. The CS– refers to the flavored saccharin solution used in training and testing. All testing took place in the rat's home cage during the mid-light phase of the light:dark cycle. Two weeks before testing commenced, the rats were placed on a food restriction schedule that maintained their body weights at 85-90% of their *ad libitum* level. The rats were initially trained to drink an unflavored 0.2% saccharin solution from sipper tubes during daily 0.5-h sessions. The sipper tube was mounted on the front of the cage held by a taut steel spring, and was positioned 3-6 cm above the cage floor. This training procedure was repeated daily until all rats approached the sipper tubes with short (< 1 min) latency, typically within three days. The limited food rations were given 1 h after each training session.

4.3. LH D1 and D2 Antagonists and Acquisition of Fructose-CFP Procedures

Four separate groups of rats were matched for their intakes of an unflavored 0.2% saccharin solution prior to training. Each rat was given eight 1-bottle training sessions (60 min/day) with the CS+/F solution presented on odd-numbered sessions, and the CS– solution presented on even-numbered sessions. A 1-day break was placed between each of the four pairs of training trials to reduce the impact of repeated bilateral LH microinjections. Training intakes were limited to 16 ml/session to minimize the difference between CS+/F and CS– intakes. Ten min prior to each one-bottle training session, rats were given bilateral mid-caudal LH microinjections (0.5 μ l/side) through a stainless steel internal cannula (33-gauge, Plastics One) that extended 1.0 mm beyond the tip of the guide cannula. This was accomplished using a Hamilton microsyringe that was connected by polyethylene tubing to the internal cannula. The four groups received vehicle (Veh: 0.5 μ l/side, n=9), SCH at doses of 12 (SCH12: 6 nmol/side, n=10) or 24 (SCH24: 12 nmol/side, n=11) nmol, or RAC at a dose of 24 nmol (RAC24: 12 nmol/side, n=11). The 12 nmol SCH dose was identical to that previously employed in the IG glucose acquisition testing in the NAcS, AMY, mPFC and LH (Touzani et al., 2008, 2009a, 2009b, 2010). The 24 nmol doses of SCH and RAC were identical to that previously employed in the fructose-CFP acquisition testing in the NAcS, AMY and mPFC (Malkusz et al., 2012). On the last two training days, the rats had access to a second sipper tube containing water, thereby familiarizing them to the presence of two sipper tubes used during the choice tests; water intake was negligible in these training trials. The left-right position of the CS and water sipper tubes was counterbalanced over these two days and into testing. Following training, the groups were given six daily 2-bottle choice

sessions (60 min/day) with unlimited (50 ml) access to the CS+/s and CS-/s solutions; no drugs were administered prior to these sessions. Solution intakes during the training and testing were measured by weighing (to the nearest 0.1 g) the bottles before and after the sessions.

4.4. LH D1 and D2 Antagonism and Expression of Fructose-CFP Procedures

Rats received ten 1-bottle training sessions (30 min/day) with 16 ml of the CS+/F solution presented on odd-numbered days, and 16 ml of the CS- solution presented on even-numbered days. On days 9 and 10, the rats had access to a second sipper tube containing water, again familiarizing them to the presence of two sipper tubes used during the choice tests; water intake was negligible in these training trials. Training intakes were limited to 16 ml/session to minimize the difference between CS+/F and CS- intakes. The left-right position of the CS and water sipper tubes was counterbalanced over these two days and into testing. Following training, the rats were given eight 2-bottle choice test sessions (30 min/day) with unlimited (50 ml) access to the CS+ and CS- solutions. Solution intakes during the training and testing were measured by weighing (to the nearest 0.1 g) the bottles before and after the 30-min sessions.

Ten min prior to the first two sessions of 2-bottle tests, all rats were given a vehicle (0.5 ul of 0.9% saline) injection. These two conditions were always conducted first to insure that all of the rats in the expression study displayed preferences that were comparable to each other; this approach has been taken by our laboratory in all previous studies (see reviews: Scalfani et al., 2011; Touzani et al., 2010b). Based on their CS+ and CS- intakes in this vehicle test, the rats were divided into two matched groups with six rats (D1 group) receiving the D1-like antagonist, SCH (Sigma Chemical Co.) at total doses of 12 (6 nmol/side), 24 (12 nmol/side) and 48 (24 nmol/side) nmol administered into the mid-caudal LH, and six rats (D2 group) receiving the D2-like antagonist, RAC (Sigma Chemical Co.) at total doses of 12, 24, and 48 nmol. Half of the rats in each group were tested with an ascending drug dose order, and the remaining rats were tested with a descending drug dose order to control for drug dose order effects. These dose ranges were employed previously in the NAcS (Bernal et al., 2008), AMY (Bernal et al., 2009) and mPFC (Malkusz et al., 2012) studies. The rats were tested twice at each drug dose with the left-right position of the CS+ and CS- solutions counterbalanced across sessions to control for position effects. A one-day rest period separated each pair of drug doses for both groups.

4.5. Data analysis

In the acquisition studies, training intakes were averaged over the 4 CS+/F and 4 CS- sessions and were analyzed with ANOVA (group $\times$ CS). Intakes during the preference tests were averaged over sessions 1-2, 3-4, and 5-6 (referred to as Tests 1, 2, and 3) to control for side position effects. A three-way randomized-blocks ANOVA compared the CS intakes of the SCH12, SCH24, RAC24 and Veh groups (Group $\times$ CS $\times$ Test). Separate two-way ANOVAs evaluated total CS intakes and percent CS+ intakes of the two groups. In the expression studies, training intakes were averaged over the five CS+/F and five CS- sessions and evaluated with a t-test. Intakes during the preference tests were averaged over the two sessions at each dose and evaluated with two-way repeated-measures analyses of variance (ANOVA, CS condition vs. Dose) for the D1 and D2 groups, respectively. Separate ANOVAs evaluated total intakes and percent CS+ intakes as a function of dose for the two groups. When main or interaction effects were found, Bonferroni corrected comparisons ($p < 0.05$) detected significant effects. When interaction effects were not observed, planned *post hoc* comparisons were utilized.

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Highlights of “Effect of dopamine D1 and D2 receptor antagonism in the lateral hypothalamus on the expression and acquisition of fructose-conditioned flavor preference in rats” by Amador et al.

- Fructose-conditioned flavor preferences (CFP) are mediated by dopamine (DA).
- Lateral hypothalamic (LH) DA D1 antagonism reduced fructose-CFP expression.
- LH DA D2 antagonism failed to affect fructose-CFP expression.
- Neither LH DA D1 nor D2 antagonism affected fructose-CFP acquisition.

Figure 1.

Histological verification of bilateral representation of mid-caudal LH cannula sites (n = 106) of 53 animals receiving SCH (n=6) or RAC (n=6) in the fructose-CFP expression study (squares) or receiving vehicle (n=9), SCH at 12 (n=10) or 24 (n=11) nmol or RAC at 24 nmol (n=11) during training in the fructose-CFP acquisition study (circles). All cannulae in both paradigms were located within the mid-caudal LH at the level of the hypothalamic ventromedial and dorsomedial nuclei, median eminence and arcuate nuclei. The schematics are derived from the stereotaxic atlas of Paxinos and Watson (2009) on plates 55, 58 and 61.

Figure 2. LH D1 and D2 Antagonists and Acquisition of Fructose-CFP: Intakes (mean $\pm$ SEM, g/60 min) of CS+ and CS- solutions in three pairs of two-bottle preference tests in animals that received mid-caudal LH microinjections of vehicle (Panel A), SCH at 12 nmol (Panel B), SCH at 24 nmol (Panel C) or RAC at 24 nmol (Panel D) 10 min prior to each training trial. Significant differences are denoted between CS+ and CS- intake within an injection condition (*) as well as antagonist-induced differences relative to corresponding vehicle treatment (+). The percentages of CS+/s intake over total intake are denoted above each pair of values.

Figure 3.

LH D1 and D2 Antagonism and Expression of Fructose-CFP: Intakes (mean $\pm$ SEM, g/30 min) of CS+ and CS- solutions in two-bottle tests in animals receiving bilateral microinjections of the DA D1 antagonist, SCH (Panel A) or the DA D2 antagonist, RAC (Panel B) at total doses of 0, 12, 24 or 48 nmol into the mid-caudal LH 10 min prior to testing. Significant differences are denoted between CS+ and CS- intake within an injection condition (*). The percentages of CS+ intake over total intake are denoted above each pair of values; significant differences relative to vehicle treatment are denoted (+).