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Neuropharmacology of Learned Flavor Preferences

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Abstract

Innate and learned flavor preferences influence food and fluid choices in animals. Two primary forms of learned preferences involve flavor-flavor and flavor-nutrient associations in which a particular flavor element (e.g., odor) is paired with an innately preferred flavor element (e.g., sweet taste) or with a positive post-oral nutrient consequence. This review summarizes recent findings related to the neurochemical basis of learned flavor preferences. Systemic and central injections of dopamine receptor antagonists implicate brain dopamine signaling in both flavor-flavor and flavor-nutrient conditioning by the taste and post-oral effects of sugars. Dopamine signaling in the nucleus accumbens, amygdala and lateral hypothalamus is involved in one or both forms of conditioning and selective effects are produced by D1-like and D2-like receptor antagonism. Opioid receptor antagonism, despite its suppressive action on sugar intake and reward, has little effect on the acquisition or expression of flavor preferences conditioned by the sweet taste or post-oral actions of sugars. Other studies indicate that flavor preference conditioning by sugars is differentially influenced by glutamate receptor antagonism, cannabinoid receptor antagonism and benzodiazepine receptor activation.

1. Introduction

In selecting which foods to eat or reject, animals (including humans) are guided by innate and acquired preferences for and aversions to flavors (combinations of taste, odor and texture stimuli). Examples are the acceptance of sweet foods and rejection of bitter foods by many species. With experience, animals refine their preferences and aversions as they associate the flavors of specific foods with the foods' postingestive consequences. Other important influences on food choice not discussed here include social and cultural factors (Blake, 2004). Much research has focused on conditioned flavor aversions which readily develop when animals experience gastrointestinal malaise after consuming a new food or fluid (Reilly and Schachtman, 2008). It is now firmly established that animals also develop strong conditioned flavor preferences (CFP) based on positive oral and post-oral associations (Sclafani, 2004). These learned flavor preferences can enhance the hedonic and incentive values of food reward (Myers and Sclafani, 2001; Sclafani and Ackroff, 2006) and are mediated in part by brain

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neurochemical systems that are implicated in innate taste preferences and drug rewards. This review summarizes recent findings on the neuropharmacology of flavor preference learning in rats.

1.1. Conditioned flavor preferences

Flavor preference learning involves multiple learning processes although is usually interpreted as 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). Flavor-flavor CFP refers here to the process by which a preference develops for a target flavor (e.g., cherry flavor) that is mixed with an already preferred flavor (e.g., sweet taste) (Sclafani, 2004). Flavor-nutrient CFP refers here to the process by which a preference develops for a target flavor that is paired with the post-oral actions of a nutrient (e.g., sucrose) (Sclafani, 2004). During normal feeding, both types of learning processes usually operate, i.e., flavor-flavor/nutrient conditioning. For example, a child eating a ripe strawberry for the first time will learn to associate the fruit's unique flavor with its sweet taste and the post-oral actions of the fruit's sugar and other nutrients. Flavor-flavor and flavor-nutrient CFPs can be separated in the laboratory and both warrant investigation because they appear to be mediated to some extent by separate neural mechanisms (see below).

1.1.1. Flavor-flavor learning—In our neuropharmacological studies, we have investigated flavor-flavor conditioning 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. Flavor preferences were then assessed by two-bottle choice tests with the CS+ and CS- flavors presented in identical solutions (e.g., both in saccharin). Saccharin was included in the CS- solution during training and in the CS+ and CS- solutions during testing because hungry rats do not drink unsweetened fruit-flavored drinks which have a sour taste. In initial studies sucrose was the US (Yu et al., 1999; Yu et al., 2000a; Yu et al., 2000b). In order to insure that it was sucrose's sweet taste rather than its post-oral actions that reinforced the flavor preference, the animals were trained and tested using a gastric ‘sham feeding’ procedure in which the ingested sucrose drained out an open gastric fistula. In subsequent studies (Baker et al., 2003; Baker et al., 2004), fructose was used as the US and the rats ‘real-fed’ the sugar, i.e., without an open gastric fistula. Although the animals were exposed to post-oral actions of the fructose, several studies have demonstrated that these actions do not reinforce flavor preferences. That is, rats do not learn to prefer a flavored saccharin solution paired with an intragastric (IG) fructose infusion when trained in short daily training sessions (Sclafani et al., 1999). Consequently, preferences for flavors added to fructose solutions could be attributed to the sugar's sweet taste. This non-invasive training procedure produces robust and persistent flavor preferences and has been adopted by other laboratories (Dwyer, 2009; Golden and Houpt, 2007).

1.1.2. Flavor-nutrient learning—Our initial studies of flavor-nutrient conditioning (or post-oral consequence learning) also used sucrose as the US (Azzara et al., 2000; Azzara et al., 2001). To ensure that the flavor preferences were conditioned by the post-oral actions rather than the taste of the sucrose, the sugar was infused IG as the animal consumed a CS+ flavored saccharin solution. Subsequent studies used glucose rather than sucrose as the US because glucose, like fructose, is a monosaccharide with equivalent osmotic effects, but is very effective in supporting post-oral flavor conditioning (Sclafani et al., 1993). In alternative one-bottle training sessions, the rats consumed a CS- flavored saccharin solution that was paired with IG water infusions. Preference conditioning was then evaluated in two-bottle tests with the CS+ and CS- flavored saccharin solutions no longer paired with IG infusions.

With our test procedures, flavor preferences are considered to be conditioned when the intake of the CS+ significantly exceeds that of the CS- in the two-bottle choice test. The magnitude of the preference is evaluated as a percentage score (CS+ intake/total intake × 100) and is statistically compared to the control group in acquisition experiments and the vehicle test in expression experiments. A potential confound in expression tests is that high doses of antagonist drugs may suppress total solution intakes such that CS+ and CS- intakes cannot differ due to floor effects. These cases are indicated in the text.

2. Dopamine and flavor preference learning

There is substantial evidence that brain dopamine (DA) systems are involved in food reward and motivation (see reviews by Berridge and Robinson, 1998; Smith, 2004; Wise, 2008). DA receptor antagonists reduce the intake of nutritive solutions (sucrose, corn oil) (Geary and Smith, 1985; Weatherford et al., 1990; Xenakis and Sclafani, 1981), and consumption of these nutritive substances increases DA efflux in several cortical and subcortical areas of the brain (Bassareo et al., 2002; Hajnal et al., 2004; Liang et al., 2006). Brain DA circuits are also involved in the mediation of food-related learning including conditioned place preference (Ågmo et al., 1995), appetitive Pavlovian conditioning (Di Ciano et al., 2001), appetitive instrumental learning (Smith-Roe and Kelley, 2000) and taste aversion learning (Caulliez et al., 1996; Fenu et al., 2001). A potential role of DA signaling in flavor-nutrient preference learning was first suggested by the finding that a CS+ flavor that had been paired with IG maltodextrin infusions elicited an increase in DA efflux in the ventral striatum (Mark et al., 1994). An early study implicating DA in flavor-flavor learning trained rats to drink differently flavored sucrose solutions following systemic injections of saline or the DA D2-like receptor antagonist, raclopride (Hsiao and Smith, 1995). To minimize post-oral effects, the one-bottle training sessions were limited to 5 min and intakes of the two sucrose solutions were yoked. In a subsequent two-bottle choice test, the rats preferred the saline-paired flavor to the raclopride-paired flavor. The authors concluded that D2-like receptor antagonism reduced the reward potency of sweet taste. Based on these findings, we further explored the role of DA signaling in the orosensory and post-oral components of sugar-conditioned flavor preferences.

2.1. Dopamine antagonism attenuates the acquisition and expression of flavor-flavor learning

We initially investigated flavor-flavor learning using the sham-feeding procedure described above (Yu et al., 2000a; Yu et al., 2000b). Rats were trained to sham-feed a CS+ flavor mixed into 16% sucrose solution and a CS- flavor mixed into a less preferred 0.2% saccharin solution. In a subsequent choice test the CS+ flavor was preferred to the CS- flavor following saline treatment. Systemic treatment with D1-like (SCH23390) or D2-like (raclopride) receptor antagonists (50-800 nmol/kg) prior to the choice tests attenuated the expression of the CS+ flavor preference at lower doses and blocked it at higher doses (400-800 nmol raclopride, 200-800 nmol SCH23390) . This systemic D1 and D2 antagonist dose range paralleled that employed previously in preference learning (Hsiao and Smith, 1995), and produced dose-dependent effects upon sweet solution intake. In a second study, rats treated with SCH23390 or raclopride (200 nmol/kg) during one-bottle training sessions subsequently displayed modest but significant (66-69%) CS+ preferences during two-bottle tests comparable to yoked control rats (72%) that had their CS training intakes limited to that of the drug groups (Yu et al., 2000b). Note that with this sham-feeding procedure, the drug and control animals consumed substantially more of the CS+ sucrose solution than the CS- saccharin solution during training, and their greater familiarity with the CS+ flavor may have contributed to their acquisition of the flavor preference.

In a follow-up study, we investigated the role of DA in flavor-flavor conditioning using the fructose real-feeding paradigm (Baker et al., 2003). The rats were trained to consume similar

amounts of a CS+ flavored 8% fructose + 0.2% saccharin solution and a less preferred CS- flavored 0.2% saccharin solution. Two-bottle tests were then conducted with both flavors presented in 0.2% saccharin solutions. Systemic treatment with SCH23390 (200 nmol/kg) or raclopride (200 nmol/kg) during training blocked the acquisition of the fructose-conditioned flavor preference (46 and 56% CS+ preference, respectively) compared to yoked control animals (66 and 75%). SCH23390 (50-800 nmol/kg) treatment at the time of two-bottle testing blocked the expression of the fructose-CFP in control animals where as raclopride blocked the preference at only one dose (200 nmol/kg). These findings indicate that the acquisition of sweet taste-conditioned flavor preferences depends upon both D1- and D2-like receptor signaling when CS+ and CS- training intakes are equated. The full expression of a previously learned CS+ preference also requires D1 and to a lesser degree D2 signaling.

2.2. Dopamine antagonism attenuates the acquisition but not expression of flavor-nutrient learning

To investigate drug effects on flavor-nutrient learning, we trained rats to drink a CS+ flavored saccharin solution paired with IG infusions of 16% sucrose and a CS- flavored saccharin solution paired with IG water infusions (Azzara et al., 2001). Rats treated with SCH23390 (200 nmol/kg) during training failed to prefer the CS+ to the CS- in the two-bottle choice test (50% CS+), whereas the yoked control rats exhibited a significant preference for the sucrose-paired flavor (72%). In contrast, the expression of CS+ preference of control rats (80%) was not blocked by the 200 nmol/kg dose of SCH23390 (76%) although the preference was attenuated (68%) at a higher dose (400 nmol/kg). Treatment of other rats with raclopride (200 or 400 nmol/kg) failed to prevent either the acquisition or expression of a CS+ preference. These finding indicate that, unlike flavor-flavor learning, flavor-nutrient learning with sugars is critically dependent only on D1-like receptor signaling. In this respect, flavor-nutrient learning is similar to flavor-toxicosis learning (with lithium chloride), which is also disrupted by D1-like but not D2-like receptor antagonists (Fenu et al., 2001). D2-like receptor antagonists may interfere with flavor-flavor learning because they interfere with the processing of orosensory (e.g., taste) rewards (Hsiao and Smith, 1995).

2.3. Brain sites mediating dopamine involvement in sugar-conditioned flavor preferences

There is an extensive literature on the critical role of the mesocorticolimbic DA system in reward processes and reward-related learning (Berridge and Robinson, 1998; Smith, 2004; Wise, 2008). In this system, DA neurons located in the ventral tegmental area (VTA) project to cortical and limbic structures including the nucleus accumbens (NAc), amygdala (AMY) and the medial prefrontal cortex (mPFC) (Swanson, 1982). Another brain DA system implicated in flavor learning (Caulliez et al., 1996) includes the A13 DA neurons located in the zona incerta that innervate the LH (Wagner et al., 1995). We recently demonstrated that DA transmission within the NAc, AMY, and LH is differentially involved in sugar-conditioned flavor preferences.

2.3.1. Nucleus accumbens—Several lines of evidence implicate DA transmission within the NAc in food reward processing and learning. Highly palatable foods (sucrose, corn oil) stimulate DA efflux in the NAc (Hajnal et al., 2004; Liang et al., 2006) and administration of DA receptor antagonists within the NAc impairs learning in several paradigms such as Pavlovian approach conditioning to a CS paired with sugar (Parkinson et al., 2002), operant responding for sugar (Smith-Roe and Kelley, 2000) and conditioned avoidance of sweet taste (Fenu et al., 2001). Collectively these findings suggest a role of DA transmission within the NAc in sugar-conditioned flavor preferences.

We examined the effect of D1-like receptor antagonism in the NAc on the acquisition and expression of flavor preferences conditioned by the post-oral reinforcing effects of glucose

(Touzani et al., 2008). Only D1-like receptor signaling was investigated in this and subsequent central studies because systemic D2-like receptor antagonism failed to impair flavor-nutrient preference conditioning by IG sugar (Azzara et al., 2001). In the first experiment, rats fitted with gastric catheters and bilateral injection cannulae in the NAc shell were trained to consume a CS+ flavor paired with IG infusions of 8% glucose solution and a CS- flavor paired with IG water in alternating one-bottle sessions. They were then given two-bottle choice tests with the CS+ vs. CS- (no IG infusions) following bilateral NAc microinjections of 0, 12, 24 and 48 nmol SCH23390 (doses are expressed as total nmol/brain). This range was chosen because it produced dose-dependent reductions in the intake of a flavored saccharin solution (Touzani and Sclafani, unpublished findings). In the choice test following vehicle (0 nmol) the rats exhibited a strong (92%) preference for the CS+ over CS- flavor. Administration of SCH23390 in the NAc shell did not significantly reduce the expression of CS+ preference at the two lower doses but blocked it at the highest dose that also greatly suppressed overall CS intakes. In two subsequent experiments, new groups of rats were fitted with bilateral cannulae in the NAc shell or core. The rats received bilateral injections of vehicle or 12 nmol of SCH23390 (a dose that did not block the expression of the CS+ preference) prior to daily one-bottle training sessions with the CS+ and CS-. The CS intakes of the vehicle control groups were limited to those of the SCH-treated rats during training. CS+ and CS- intakes were paired with 8 ml IG infusions of 8% glucose and water, respectively, during training and no brain injections or IG infusions were made during the subsequent two-bottle choice tests. In the choice tests, the SCH rats failed to display significant CS+ flavor preferences (shell group 61%, core group 55%) unlike the robust preferences displayed by the vehicle control groups (83%, 89%). These findings demonstrate that activation of D1-like receptors in both the NAc shell and core is critical for the acquisition but not the expression of a flavor-nutrient preference conditioned by IG glucose.

In a parallel flavor-flavor study, we trained rats to associate a CS+ flavor with the taste of an 8% fructose + 0.2% saccharin solution and a CS- flavor with the less preferred taste of a 0.2% saccharin solution (Bernal et al., 2008). In subsequent CS+ vs. CS- choice tests with both flavors presented in saccharin only, the rats displayed a significant 75% CS+ preference when treated with vehicle. Administration of SCH23390 or raclopride (12, 24 and 48 nmol/brain) in the NAc shell attenuated the expression of the fructose-CFP at the two highest doses. In a second experiment, NAc shell administration of 12 nmol SCH23390 or raclopride during training did not prevent the rats from acquiring a significant fructose-CFP (70%, 73%), but resulted in a more rapid extinction of this preference with repeated testing compared to rats treated with vehicle during training. Thus, DA transmission in the NAc shell is partially involved in the expression and acquisition of a flavor-flavor preference but the acquisition effect is less pronounced than that observed with flavor-nutrient conditioning (Touzani et al., 2008).

2.3.2. Amygdala—There is extensive evidence implicating the AMY in motivation and reward-related learning (Baxter and Murray, 2002; Cardinal et al., 2003) and recent lesion studies provide evidence that the AMY is involved in flavor preference learning induced by both the sweet taste and the post-oral reinforcing properties of nutrients (Gilbert et al., 2003; Touzani and Sclafani, 2005). The AMY receives DA innervation from VTA neurons and contains moderate to high densities of D1-like and D2-like receptors (Asan, 1997; Mansour et al., 1990). Activation of D1-like receptors in the AMY is required for learning a sucrose-reinforced bar pressing response (Andrzejewski et al., 2005), and neurochemical studies report that feeding, gastric nutrient loads or stimuli predicting food promote DA efflux in the AMY (Hajnal and Lenard, 1997; Harmer and Phillips, 1999).

To test the hypothesis that activation of D1-like receptors in the AMY is involved in flavor preference learning, we determined the effects of SCH23390 injections in the AMY on the expression of flavor preferences conditioned by IG glucose (Touzani et al., 2009a). Following

training, the rats displayed a strong CS+ preference (89%) when vehicle was injected into the AMY. AMY injections of SCH23390 (12, 24 and 48 nmol/brain) produced dose-related reductions in CS+ intake and blocked the CS+ preference at the two highest doses that also greatly suppressed CS intakes. In an acquisition experiment, rats given AMY injections of SCH23390 (12 nmol/brain) during training failed to display a significant CS+ preference (55%) in the drug-free two-bottle choice test unlike the robust 81% preference displayed by the vehicle-treated rats. Additional experiments employed the same acquisition procedure except that brain injections targeted the basolateral (BLA) or central nuclei (CeA) of the AMY. SCH23390-treated rats learned to prefer the CS+ to the CS-, but their preferences (BLA: 59%, CeA: 73%) were weaker than those displayed by respective vehicle control rats (80%, 88%). Thus, antagonism of D1-like receptors in the AMY during training blocked the acquisition of a flavor-nutrient preference at a SCH23390 dose (12 nmol) that did not prevent the expression of a previously learned preference. Because antagonism of the D1-like receptors in either the BLA or CeA during training only attenuated the acquisition of the glucose-CFP, it appears that activation of D1-like receptors in the entire AMY is crucial for flavor preference conditioning by IG glucose. Consistent with these results, we previously reported that large AMY lesions blocked flavor preference conditioning by IG glucose whereas preference conditioning was only attenuated by selective BLA lesions (Touzani and Sclafani, 2005).

In a parallel flavor-flavor conditioning study, Bernal et al. (2009) observed that the expression of a fructose-based CS+ preference (77%) after vehicle treatment in the AMY was dose-dependently attenuated, but not eliminated by AMY injections of SCH23390 (71 to 66%) or raclopride (76% to 68%) at doses of 12 to 48 nmol. AMY injection of SCH23390 (12 nmol) during flavor conditioning did not prevent the rats from acquiring a fructose-CFP (70%), but resulted in a more rapid extinction of the preference with repeated testing. In contrast, raclopride treatment during training did not block CS+ flavor conditioning nor promote extinction of the preference. Thus, SCH23390 but not raclopride had similar effects on the flavor-flavor learning when injected into the AMY and NAc. The lack of D1-like and D2-like receptor antagonism in either the NAc or AMY to prevent the acquisition of flavor-flavor preference conditioning by fructose suggests that simultaneous DA transmission in these two structures is required to promote this kind of learning.

These data suggest a distributed brain DA network mediating flavor preference conditioning particularly with regard to D1-like receptors. In this network, cortical and forebrain structures such as the AMY and NAc receive dense DA projections from the A10 cell group of the VTA (Swanson, 1982) and these structures are interconnected via glutamatergic fibers (Brog et al., 1993; Christie et al., 1987). This network has also been proposed in food reward-based instrumental learning (Andrzejewski et al., 2005; Baldwin et al., 2002). The NAc receives major glutamatergic inputs from the AMY (Brog et al., 1993; McGeorge and Faull, 1989; Zahm, 2000), and functional NAc-AMY interactions have recently been proposed for forms of incentive learning (Ambroggi et al., 2008; Di Ciano and Everitt, 2004; Setlow et al., 2002; Simmons and Neill, 2009). Interestingly, DA released in the NAc facilitates firing of neurons elicited by these glutamatergic inputs (Nicola, 2007), and repeated tetanizations of either the NAc or AMY neurons induces long-term potentiation that is modulated by DA D1-like receptors (Bissiere et al., 2003; Loretan et al., 2004; Schotanus and Chergui, 2008). Thus, as proposed by Beninger (1993) and Wickens (1993), it is possible that DA released in the AMY and NAc by food or food-associated cues, and acting on D1-like receptors, promotes flavor preference learning by strengthening the effectiveness of activated glutamatergic synapses in these structures. This DA signaling may underlie different processes that develop according to a functional hierarchy within the mesolimbic network. Indeed, evidence indicates that AMY and NAc neurons respond to reward-predictive cues, that NAc neuronal responses to these predictive cues require excitatory projections from the AMY (Ishikawa et al., 2008), and that AMY neuronal activation evoked by predictive cues precedes that of NAc neurons (Ambroggi

et al., 2008). AMY DA signaling, via the D1-like receptors, may be involved in flavor preference learning by strengthening the association between the predictive flavor cue and the affective significance of food reward (Balleine and Killcross, 2006). NAc DA signaling, via the D1-like receptors, may be involved in flavor preference learning based on associations between the flavor cue and the outcome of its consumption (stimulus-outcome association) as well as in the execution of actions upon the presentation of the predictive cue (stimulus-action association) (Ikemoto, 2007).

2.3.3. Lateral hypothalamus—The LH not only plays an important role in the control of food intake (Berthoud, 2002), it is also implicated in flavor preference and aversion learning (Touzani and Sclafani, 2001; Touzani and Sclafani, 2002). Furthermore, D1-like signaling in the LH modulates flavor aversion learning (Caulliez et al., 1996; Fenu et al., 2001). We therefore examined the effect of D1-like receptor antagonism in the LH on flavor preference conditioning by IG glucose infusion (Touzani et al., 2009b). LH infusions of SCH23390 (12 nmol/brain) during training did not prevent the acquisition of a IG glucose-CFP, but the CS+ preference was attenuated compared to that displayed by a vehicle control group (61 vs. 87%). The established CS+ preference (88%) of the control rats remained unchanged (90%) when the rats were injected with SCH23390 (12 nmol) prior to testing. Thus, D1-like receptor signaling in the LH contributes to the acquisition of a flavor-nutrient preference, but not the expression of previously learned preference. The effects of DA antagonism in the LH on fructose-conditioned flavor-flavor preferences are under investigation.

3. Opioids and flavor preference learning

The involvement of brain opioid systems in flavor preference learning has also been investigated in our laboratory. It is well established that general and selective opioid receptor antagonists suppress food and fluid intake whereas opioid agonists increase food and fluid intakes in a variety of situations (Bodnar, 2004; Cooper, 2007; Levine, 2006). Of particular relevance here are findings implicating opioid signaling in the ingestive response to palatable foods and fluids. For example, the opioid antagonist naloxone reduced the intake of sweet solutions more than that of plain water in one-bottle drinking tests (Sclafani et al., 1982), blocked the preference for a saccharin solution over water in two-bottle tests (Cooper, 1983; Le Magnen et al., 1980), reduced sugar solution intakes in sham-feeding tests that minimized post-oral factors (Kirkham and Cooper, 1988; Rockwood and Reid, 1982), and suppressed hedonic taste reactivity responses to intraoral sugar infusions (Parker et al., 1992). Such data predict that opioid antagonists should reduce flavor preference conditioning by the sweet taste of sugars.

An early study by Mehiel (1996) appeared to provide such evidence. Rats were trained to drink a CS+ flavor mixed in a 10% glucose solution and a CS- flavor mixed in a less preferred 0.25% saccharin solution. Animals injected with saline prior to glucose training sessions displayed a strong CS+ preference in subsequent CS+ vs. CS- choice tests, whereas rats injected with naloxone failed to acquire a CS+ preference. A problem with this experimental procedure, however, is that only the CS+ flavor was paired with the naloxone during training and therefore the rats may have failed to develop a CS+ preference because they associated the flavor with possible aversive effects of the drug. We therefore conducted follow-up studies in which the opioid antagonist naltrexone was paired with both CS+ and CS- flavors during training. In addition, separate studies determined drug effects on the flavor-flavor and flavor-nutrient conditioning effects of sugars. Naltrexone was chosen because although it possessed the same affinity and potency for opioid receptors as naloxone, it possesses a longer duration of pharmacodynamic action than naloxone, and was therefore more appropriate to the time courses employed in the present series of studies (Archer, 1981).

3.1. Opioid antagonism fails to disrupt the acquisition or expression of flavor-flavor learning

We first examined the effects of naltrexone on the expression of a flavor preference conditioned by the sweet taste of sham-fed sucrose (Yu et al., 1999). Following training, the rats significantly preferred the CS+ to CS- flavor in two-bottle tests and treating the rats with naltrexone (0.1 - 10 mg/kg) prior to the choice tests failed to block the CS+ preference. This systemic dose range of naltrexone has been shown to effectively reduce intakes of sugars and saccharin (Bodnar, 2004). In an acquisition study, rats were injected with naltrexone (0.1 mg/kg) or saline prior to CS+ and CS- training sessions. Although naltrexone treatment suppressed CS+ intakes during training, it did not prevent the rats from displaying a CS+ preference in the drug-free two-bottle test comparable to that displayed by the vehicle control rats (89 vs. 86%).

In a subsequent study we determined the effects of an expanded naltrexone dose range (0.1, 1 and 5 mg/kg) on preference conditioning by the sweet taste of fructose (Baker et al., 2004). Although naltrexone treatment dose-dependently reduced CS intakes during one-bottle training, it did not prevent flavor preference conditioning. The CS+ preferences of the three naltrexone groups (72-86%) did not significantly differ from that of the saline-treated group (78%). In addition, naltrexone injections prior to two-bottle choice tests did not significantly reduce the expression of the previously learned CS+ preference. These results taken with the sucrose sham-feeding data indicate that endogenous opioids are not intimately involved in flavor preference conditioning by the sweet taste of sugar.

3.2. Opioid antagonism fails to disrupt the acquisition or expression of flavor-nutrient learning

In another study we investigated the effects of systemic naltrexone (0.1 or 1 mg/kg) treatment on flavor conditioning by IG sucrose infusions (Azzara et al., 2000). Although naltrexone reduced training intakes of the CS solutions, it did not prevent the animals from acquiring a significant CS+ preference. In particular, rats treated with 1 mg/kg naltrexone or vehicle during training displayed 88% and 90% preferences for the CS+, respectively, during the drug-free two-bottle choice test. Furthermore, injecting the rats with naltrexone (0.1 - 10 mg/kg) prior to two-bottle testing failed to reduce the expression of a previously learned CS+ preference. These finding indicate that flavor preference learning based on the post-oral reinforcing properties of sugar, like flavor-sweet taste learning, does not depend upon opioid receptor signaling.

3.3. Opioid antagonism in the NAc fails to disrupt the expression of sugar-conditioned flavor preferences

It is possible that the systemic naltrexone injections in our studies, by affecting opioid receptors in various brain regions, obscured the contribution of brain opioid signaling to flavor preference conditioning (Gosnell and Levine, 2009). This is suggested by a recent study (Woolley et al., 2006) reporting that systemic treatment with naltrexone reduced the intakes of both preferred (chocolate-flavored) and less preferred (banana-flavored) food pellets in a choice test, whereas naltrexone injections into the NAc selectively reduced the intake of the preferred chocolate-flavored food. We therefore determined the effects of NAc shell or core injections of naltrexone on the expression of CS+ flavor preferences conditioned by the sweet taste of fructose or the post-oral actions of glucose (Bernal et al., 2010). Following flavor conditioning, the rats received bilateral injections of saline or naltrexone (1, 25 and 50 μg/brain) in the NAc, 10 min prior to the two-bottle CS+ vs. CS- tests. This dose range of accumbal naltrexone includes the high dose used by Woolley et al. (2006). Overall, the NAc naltrexone injections produced little or no reduction in the CS+ preferences conditioned by oral fructose or IG glucose. Thus, NAc core or shell injections of naltrexone were no more effective than systemic injections in attenuating the expression of sugar-conditioned flavor preferences.

3.4 Opioid signaling and conditioned vs. unconditioned flavor preferences

The failure of naltrexone treatment to block the acquisition or expression of sugar-conditioned preferences would appear to conflict with the many reports that opioid antagonists reduce the intake of and preference for palatable foods and fluids (Bodnar, 2004; Cooper, 2007; Levine, 2006). These studies did not involve conditioned preferences, however, and it is possible that unlearned preferences are more dependent upon opioid signaling than are learned preferences. Alternatively, differences in testing procedures and/or flavor choices used in the various studies might account for the discrepant results. Of particular relevance to our conditioning experiments are studies reporting opioid effects on saccharin solution intake and preference. Naloxone was found to completely block the preference for a saccharin solution over water in thirsty rats (Cooper, 1983; Le Magnen et al., 1980) and to reduce saccharin intake in hungry or non-deprived rats (Gosnell and Majchrzak, 1989; Lynch and Burns, 1990; Lynch and Libby, 1983). In these latter studies, however, saccharin was apparently still preferentially consumed to water, e.g., water intake was either not reported or described as being nil. Because non-deprived or hungry rats typically drink little or no water in sweetener vs. water tests, we investigated the effects of systemic naltrexone treatment on saccharin preference by giving hungry rats the choice of two different saccharin solutions (0.20 vs. 0.15%) (Sclafani and Azzara, unpublished findings). When treated with vehicle, the rats drank substantially more of the 0.20% than 0.15% saccharin solution (11.0 vs. 1.6 g/30 min). Although 1, 2.5, and 5 mg/kg naltrexone selectively reduced the intake of the 0.20% saccharin solution to 7.0, 6.1, and 4.4 g/30 min, respectively, the rats continued to preferentially drink this concentration following drug treatment. As a result, the preference for 0.20% saccharin following vehicle (86%) and naltrexone treatments (74-85%) did not significantly differ. These effects of naltrexone on the intake of and preference for the unflavored 0.2% saccharin solution are very similar to the drug effects we observed for the preferred CS+ flavored saccharin solutions in our conditioning experiments. Thus, at least with saccharin solutions, opioid receptor antagonism does not appear to differentially alter unconditioned and conditioned preferences when test conditions are closely matched. As previously discussed (Baker et al., 2004; Yu et al., 1999), it may be that opioid antagonism would more effectively disrupt flavor conditioning when the CS flavors were paired with qualitatively different flavor USs (e.g., sweet vs. starch) rather than USs that differed only in sweet taste intensity.

4. Other neurotransmitters and flavor preference learning

4.1. Glutamate

The role of glutamate and its receptors in learning and memory has been recognized for many years. Glutamate transmission within key structures such as the NAc, AMY and mPFC is critical for reward-related learning in part by interacting with DA systems in these areas (Kelley, 2004).

The involvement of glutamate signaling in flavor preference learning is indicated by a study investigating the effects of systemic administration of the non-competitive NMDA receptor antagonist, MK-801, on flavor-flavor conditioning by fructose (Golden and Houpt, 2007). Using a conditioning procedure similar to that described above, MK-801 treatment blocked the acquisition, but not the expression of the fructose-based CFP. MK-801 substantially reduced the training intake of the CS+ fructose solution which may have contributed to the impaired flavor conditioning. The importance of glutamate signaling to flavor-nutrient learning and the central sites of action remain to be investigated.

4.2. Cannabinoids

There is an abundant literature on the role of cannabinoids in the mediation of food intake with the cannabinoid CB-1 receptor in particular is implicated in food intake and preferences

(Cooper, 2007). In addition, several lines of evidence suggest that cannabinoids interact with opioid and DA systems to promote intake of palatable food and food reward (Cooper, 2004; Cota et al., 2006; Gardner, 2005). We investigated the involvement of cannabinoid signaling in flavor conditioning by determining the effects of CB-1 receptor antagonism with AM-251 on a fructose-CFP (Miner et al., 2008). The expression of the fructose-conditioned CS+ preference (74%) was partially suppressed by systemic administration of 0.1, 1, or 3 mg/kg AM-251 (to 65 – 68%) with the effect being significant at the two lowest doses. Yet treatment with AM-251 (1 mg/kg) during CS training sessions did not significantly retard the development of a fructose-CFP. These findings suggest a limited role of CB-1 signaling in flavor-flavor preference conditioning. The role of CB-1 receptors in flavor-nutrient learning has yet to be investigated.

4.3. Benzodiazepines

Another neurotransmitter receptor implicated in flavor palatability is the benzodiazepine receptor, a part of the $GABA_A$ receptor complex (Cooper, 2005; Cooper, 2007). A recent study by Dwyer (2009) determined if midazolam, a benzodiazepine receptor agonist which increases sweet solution intake, would enhance flavor conditioning by the sweet taste of fructose. Instead, treatment with midazolam during training decreased the CS+ preference compared to vehicle-treated rats (72 vs. 87%). This was attributed to the drug enhancing the palatability of the CS- saccharin solution during training as indicated by a selective increase in CS- intake. Midazolam treatment also did not enhance the expression of the learned CS+ although it did increase over CS intakes. Midazolam also did not alter the acquisition or expression of flavor preferences conditioned by maltodextrin which appears to act by its post-oral rather than taste palatability effects. According to Dwyer (2009), the failure of midazolam to selectively enhance the CS+ preference when administered during training or testing was not surprising given the close relationship between benzodiazepine and opioid effects on food palatability and the failure of opioid antagonists to influence flavor preference conditioning (Azzara et al., 2000; Baker et al., 2004).

5. Conclusions

It has long been recognized that learning plays an important role in the establishment and strengthening of food preferences, particularly the preferences for high-fat and high-sugar foods that may promote overeating and obesity. Most research has focused on learned flavor aversions but during the last two decades there has been increasing attention to learned flavor preferences that are based on the oral and post-oral reinforcing properties of nutrients. Since food reward has been closely linked to brain opioid and DA systems, initial studies of flavor preference learning concentrated on these two systems. To date, our findings using the general opioid receptor antagonist, naltrexone, do not support significant opioid involvement in preference learning induced by either the taste or the post-oral reinforcing properties of sugars. A possible involvement of opioid signaling in preference conditioning by other nutrients (starch, fat) is under investigation. Instead, our studies reveal a critical role for DA signaling via the D1-like receptors and, to a lesser degree, D2-like receptors in sugar-conditioned flavor preferences that involves at least the NAc and AMY, and suggest the involvement of a distributed DA network in food preference learning. In this network, DA projections from the VTA densely innervate forebrain structures involved in reward, motivation and learning, and DA signaling in these structures may underlie different incentive processes that develop according to a functional hierarchy.

DA obviously does not act alone but interacts with other neurotransmitter systems such as glutamate to promote food reward learning (Kelley, 2004). During the early phases of learning (acquisition), DA signaling at the level of D1-like receptors (reward signal) may trigger a series

of molecular cascades necessary to stabilize glutamatergic synapses that carry the sensory information about foods flavors (see review: (Sutton and Beninger, 1999). Once food preferences are acquired, their expression as well as the strengthened glutamatergic synapses become independent of DA signaling. This suggests that the acquisition of food preferences depends on both DA D1-like receptors and glutamatergic receptors, whereas their expression may rely on glutamatergic receptors. A better understanding of the basic cellular and molecular mechanisms involved in appetite and learned food preferences may provide insights into the clinical treatment of overeating and obesity.

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